Supplemental Materials

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SUMMARY OF CHANGES

For Protocol Amendment to:

NCI Protocol #: 8601
Local Protocol #: OSU 10045

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#	Section	Page(s)	Change
I.	**Header**	**All**	Revised version date to May 12, 2014 for consistency with Consent form.
II.	**Cover**	**2**	Added Amendment / Version 7.0 / May 12, 2014. No other changes to Protocol.

NCI PROTOCOL #: NCI 8601

Local Protocol #: OSU 10045

A 2-arm Randomized Phase II Study of Carboplatin, Paclitaxel plus Reovirus Serotype-3 Dearing Strain (Reolysin®) vs. Carboplatin and Paclitaxel in the First Line Treatment of Patients with Recurrent or Metastatic Pancreatic Cancer

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Study Agents:

Reovirus Serotype 3- Dearing Strain: REOLYSIN®, NSC# 729968, BB-IND 13370; supplied by National Cancer Institute

Carboplatin: commercially available

Paclitaxel: commercially available

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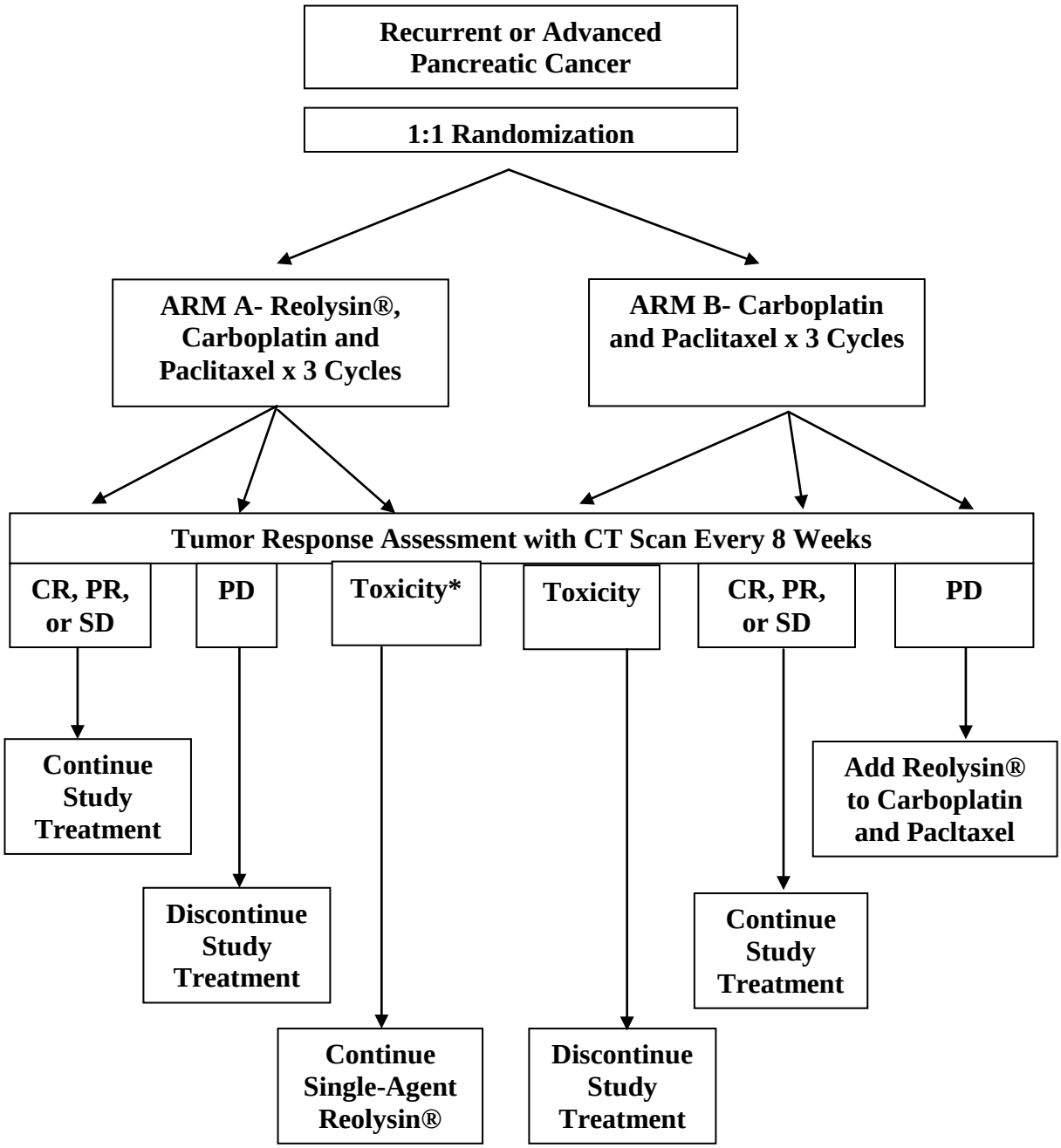
SCHEMA



*If toxicity is deemed to be related to carboplatin and/ or paclitaxel rather than Reolysin®, then treatment may continue with single agent Reolysin®.

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1 OBJECTIVES

1.1 Primary Objectives

1.1.1 To assess the improvement in progression-free survival with Reolysin®, carboplatin, and paclitaxel relative to carboplatin and paclitaxel alone in patients with recurrent or metastatic pancreatic cancer.

1.2 Secondary Objectives

1.2.1 To evaluate the safety and tolerability of Reolysin® in combination with carboplatin and paclitaxel versus without Reolysin® in patients with recurrent or metastatic pancreas cancer.

1.2.2 To compare the treatment groups for other efficacy endpoints such as overall response rate and overall survival.

1.2.3 To define how the combination of Reolysin® and CP modulate factors regulating immunity to reovirus and its persistence in the system circulation of patients with pancreatic cancer.

1.2.4 To prospectively establish and validate the relationship between Ras mutations in tumor samples and response to Reolysin

2 BACKGROUND

2.1 Pancreatic Cancer

Pancreas cancer is the fourth leading cause of cancer death in the United States, with an estimated 42,470 new diagnoses and 35,240 deaths attributable to the disease in 2009. Most pancreatic cancer is diagnosed either as locally advanced (26%) or metastatic (52%) with 5-year survival rates of less than 5% [1]. For many years, 5-fluorouracil (5-FU) was the mainstay of treatment and there has been little improvement in outcomes over the last four decades. The current standard of care for advanced pancreatic cancer is considered to be gemcitabine, based on a small phase III randomized study of 126 patients which demonstrated gemcitabine to be superior to 5-FU based on an unconventional primary endpoint of clinical benefit response (23.8% vs. 4.8%) compared with 5-FU [2]. Compared with 5-FU, gemcitabine resulted in an improved response rate (5.4% vs. 0%), median overall survival (5.7 vs. 4.4 months), and one-year survival (18% vs. 2%). When erlotinib was added to gemcitabine, the survival advantage was an added 2 weeks at the median with a statistical significance over gemcitabine alone [3]. Other studies to date that have attempted to combine agents (cytotoxic or biologic) with gemcitabine have failed to show any improvement over gemcitabine alone [4,5,6]. Recently, the ESPAC3 adjuvant study suggested no difference in survival between gemcitabine and 5-FU in patients with resected pancreas cancer, calling into question the superiority of gemcitabine [7]. While still considered a standard of care for pancreatic cancer, gemcitabine offers very limited benefit at best. Other treatment approaches are clearly needed in this desperate disease.

2.2 *KRAS* mutations in Pancreatic Cancer

Ras is a small GTP binding protein that is a common upstream molecule mediating several signalling pathways including Raf/MEK/ERK and PI3/Akt. The family of *Ras* genes is made up of three genes (*H-Ras, K-Ras, and N-Ras*), differentiated according to their homology to different sarcoma oncogenes. These *Ras* genes show varying abilities to activate the various signalling cascades. Ras proteins are located on the intracellular domain of the cell membrane and function as a switch to relay extracellular signals to the nucleus. Activation induces a change in conformation from GDP to GTP with downstream phosphorylation of proteins such as Raf, MEK, ERK and nuclear proto-oncogenes such as c-Myc, c-Fos and c-Jun [8].

Mutations that lead to the expression of constitutively active Ras protein are observed in approximately 30% of all human cancers, including more than 90% of all pancreatic ductal adenocarcinomas [9,10,11]. It is believed that activating mutations in the *K-ras* oncogene are critical for initiation of pancreatic ductal carcinogenesis. Genetically engineered mouse models in which a Cre-activated *K-Ras* (G12D) allele is knocked into the endogenous *K-Ras* locus and then crossed with mice expressing Cre recombinase in pancreatic tissue have provided valuable mechanistic insights into the role of *K-Ras* in the pathogenesis of pancreatic cancer [11].

2.3 Reovirus

Reovirus Serotype 3 – Dearing Strain (Reolysin®) is a naturally occurring, ubiquitous, non-enveloped human reovirus with a genome that consists of 10 segments of double-stranded RNA [12]. While community-acquired reovirus infection in humans is generally mild and limited to the upper respiratory and gastrointestinal tract, reovirus has been shown to replicate specifically in, and be cytopathic to, transformed cells possessing an activated Ras signalling pathway [13-17]. The specificity of the reovirus for Ras-transformed cells, coupled with its relatively nonpathogenic nature in humans, makes it an attractive anti-cancer therapy candidate.

The preferential lysis of cells with activated Ras by reovirus appears to be due to the inhibition of double-stranded RNA-activated protein kinase (PKR) in these cells [17]. In non-Ras activated cells, PKR auto-phosphorylates in the presence of viral transcripts, which activates it and results in inhibition of viral protein synthesis, thus preventing viral replication. Ras activated cells inhibit the auto-phosphorylation of PKR, keeping it in an inactive state, and allowing viral translation and eventually oncolysis to take place. In transformed cells with mutations of the Ras proto-oncogene, reovirus has been shown to possess cytopathic activity [17,18]. Ras can also be activated by upstream mitogenic signals such as tyrosine receptor kinases. Activation of the Ras/Raf/MEK2 pathway also interrupts the interferon-induced antiviral responses and allows for further viral infection [12-20].

Enhancement of oncolytic viral therapy by combination with standard chemotherapeutic agents has previously been described in the literature for a variety of viral constructs [21-

24]. The synergistic activity of reovirus, in combination with commonly used cytotoxic agents, has been established *in vivo* in human cancer cell lines and also in early clinical studies [25-27]. Reovirus has also been demonstrated to enhance the cytotoxicity of radiation in a range of tumor cell lines both *in vitro* and *in vivo* [28].

It is important to note that reovirus is a naturally occurring virus which is not genetically modified. Reovirus replication is entirely cytoplasmic and replication does not include any nuclear events. **Therefore, treatment with reovirus does not constitute gene therapy.**

To date, there have been 16 completed or ongoing clinical Phase 1 or Phase 2 studies in Canada, the United Kingdom and the United States with Reovirus Serotype 3 – Dearing Strain. More than 250 patients with malignancies including malignant gliomas, bone and soft tissue sarcomas, prostate cancer, and various advanced malignancies have been treated, receiving single or multiple doses, intratumorally or intravenously, and as monotherapy or in combination with chemotherapy or radiotherapy. In previous phase I dose escalation studies, biopsy analysis confirmed the presence of viable virus in post-treatment (but not pre-treatment) tumor samples; there was also minimal viral persistence (viral shedding) in body fluids observed in this study [27]. In the 164 patients treated with intravenous administration to date, the adverse events associated with Reolysin® have been mild to moderate in severity and have predominantly been "flu-like" in nature (such as fever, chills, headache, fatigue, rhinorrhea, and cough) as well as GI symptoms, including nausea, vomiting, and diarrhea. Moderate and transient alterations in hepatic function tests and hematologic values have also been observed [15]. Although there is a theoretical risk of viral-mediated cardiomyopathy, this has not been clinically significant in the trials to date. Of note, four phase I dose-escalation studies utilizing IV administration have been completed with no Maximum Tolerated Dose (MTD) reached at doses up to 3×10^{10} $TCID_{50}$ for up to 5 consecutive days with courses repeated every 21-28 days.

The question of immune activation has also been evaluated. An increase in neutralizing anti-viral antibodies was observed in patients with or without pre-existing immunity. In a phase I study of Reolysin® given intravenously to patients with advanced malignancies, all patients had neutralizing anti-reovirus antibodies (NARA) detectable pre-treatment, and titres increased about 1 week after treatment and with subsequent treatment courses reaching a plateau [15,27]. See Section 2.5 below for additional discussion of NARA. In addition, the US National Cancer Institute is sponsoring two ongoing trials, one in patients with melanoma and one in patients with ovarian cancer.

Recent studies in colorectal cancer patients have demonstrated that intravenously administered Reolysin® rapidly associates with the cellular components of human blood which allow it to "hitchhike" to tumor deposits and avoid immune recognition Adair et al., Sci Transl Med, 2012. This was in contrast to prior studies in animal models showing that 'naked' virus was eliminated and unable to reach the tumor site Ilett EJ et al. Gene Therapy, 2009. This unique property is promising in that it suggests viral persistence in the circulation of patients can be further enhanced by CP chemotherapy. To our knowledge, the effect of

CP on viral persistence has not been examined. We postulate that changes in the systemic profile of both NARA and the phenotypic composition of immune effector cells following CP treatment will enhance the ability of free virus to access immune cells.

Table 1 provides a summary of Oncolytics Biotech, Inc.'s clinical studies to date [15]:

Table 1: Reovirus Serotype 3 – Dearing Strain (REOLYSIN®) Clinical Trials to date

Study No.	Phase	Rx Route	Maximum Dose (TCID50)	Tumor Type	Investigator Location	Status	Outcome	Tumour Response / Evaluable
REO 001	1	ITu (Peri-cutaneous)	Escalation to 1 × 1010 (Single Dose)	Various Cancers	Don Morris Tom Baker CC Calgary, AB	Completed 19 pts	No MTD reached	3 PR (1 SCC H&N. melanomaX2) at injection site; 3 PR synchronous lesions 4 SD /18
REO 002	1	ITu	5 × 109 (Fixed) (Single Dose)	Prostate Cancer	Don Morris Tom Baker CC Calgary, AB	Completed 6 pts	No MTD reached	5 histopath. response /6
REO 003	1	ITu	Escalation to 1 × 109 (Single Dose)	Various Gliomas	Peter Forsyth Tom Baker CC Calgary, AB	Completed 12 patients (Forsyth et al., 2008)	No MTD reached	6 pts alive >6mos; 3 pts alive >12mos /12
REO 004	1	IV	Escalation to 3 × 1010 (On Day 1, Q28 days)	Various Cancers	Sanjay Goel Montefiore CC Bronx, NY	Completed 18 pts (Ghalib et al., 2009)	No MTD reached	1 PR (breast cancer), 7 SD /18
REO 005	1	IV	Escalation to 3 × 1010 (On Days 1 – 5, Q28 days)	Various Cancers	1) Johann de Bono Royal Marsden Hosp Sutton, UK 2) Hardev Pandha St. Georges Hosp London, UK and Royal Surrey Hosp Surrey, UK	Completed 33 pts (Vidal et al., 2008)	No MTD reached	8 SD including 2 minor responses (prostate and cervical) 1 pt (prostate) with 50% decrease in PSA 2 pts (colorectal) with 27-60% reduction in CEA / 22
REO 006 (with concurrent radiation)	1a	ITu (Peri-cutaneous)	Escalation to 1 × 1010 (On Days 2 & 4) with 20 Gy Rt	Various Cancers	1) Kevin Harrington Royal Marsden Hosp Sutton, UK 2) Alan Melcher St. James Hosp Leeds, UK	Completed 12 pts	No MTD reached	2 PR (esophageal, squamous skin carcinoma) 5 SD in target lesions /7

	1b	ITu (Peri cutan eous)	Escalation to 1 × 1010 (On Days 2, 4 9,11, 16, 18) with 36 Gy Rt	Various Cancers	1) Kevin Harrington Royal Marsden Hosp Sutton, UK 2) Alan Melcher St. James Hosp Leeds, UK	Completed 13 pts (Vidal et al., manuscript in preparation)	No MTD reached	4 PR (colorectal cancer, melanoma, ovarian carcinoma, melanoma) 3 SD target lesion /7
REO 007	1/ 2	ITu	Escalation to 1 × 1010 (Single dose)	Various Gliomas	1) James Markert Univ. of Alabama Birmingham, AL 2) E. Antonio Chiocca Ohio State Univ. Columbus, OH 3) John Yu Cedars-Sinai MC Los Angeles, CA	Ongoing 12 pts	No MTD to date	pending
REO 008 (with concur-rent radiation)	2	ITu (Peri-cutan eous)	1 × 1010 (Fixed) (On Days 2 & 4) With 20 Gy Rt	Various Cancers	1) Kevin Harrington Royal Marsden Hosp Sutton, UK 2) Alan Anthoney St. James Hosp Leeds, UK 3) Mark Saunders Christie Hosp Manchester, UK 4) Andrew Bateman Southampton Hosp Southampton, UK 5) Shahreen Ahmad Guys Hosp London, UK	Complete 16 pts (Saunders et al., 2009)	No DLTs	7 SD in target lesion 4 PR in target lesion (lung, melanomax2, gastric) 2 minor response in target lesion (thyroid, ovarian) /14
REO 009 (with gemcitabine)	1	IV	Escalation to 3 × 1010 (On Day 1, Q21 days)	Advanced Malignanci es	1) Johann de Bono Royal Marsden Hosp Sutton, UK 2) Jeff Evans Beatson WSCC Glasgow, UK	Completed 16 patients	Recom-mended Phase 2 dose 1 × 1010	2 PR (breast, nasopharyngeal) 5 SD /10 (disease control rate = CR+PR+SD = 70%)
REO 010 (with docetaxel)	1	IV	Escalation to 3 × 1010 (On Days 1 – 5, Q21 days)	Advanced Malignanci es	1) Hardev Pandha Royal Surrey Hosp Surrey, UK 2) James Spicer Guys Hosp London, UK 3) Andrew Protheroe Churchill Hosp Oxford, UK	Completed. 24 patients (Rudman et al., 2009)	No MTD reached	4 PR (breast, gastric) 10 SD /16 (disease control rate = CR+PR+SD = 88%)

REO 011 (with paclitaxel/ carboplatin)	1 & 2	IV	Escalation to 3 × 1010 (On Days 1 – 5, Q21 days)	Advanced Malignancies (Phase 2 arm in H&N Cancer)	1) Kevin Harrington Royal Marsden Hosp Sutton, UK 2) Geoff Hall St. James Hosp Leeds, UK	Completed 31 patients (Karapanagiotou et al., 2009)	No MTD reached	8 PR in H&N 6 SD in H&N /19 evaluable H&N (disease control rate in H&N = 74%) 1 PR +1 SD among 4 melanoma patients
REO 012 (with cyclophos-phamide)	1	IV	REOLYSIN® 3 × 1010 (Fixed) (On Days 1-5, Q28 days) Escalation of cyclophosphamide	Advanced Malignancies	1) Johann de Bono Royal Marsden Hosp Sutton, UK 2) James Spicer Guys Hosp London, UK 3) Hardev Pandha Royal Surrey Hosp Surrey, UK	Ongoing 14 patients	No MTD to date	pending
REO 013 (investigator IND)	1	IV	1 × 1010 (Fixed) For 5 days	Advanced Colorectal Cancer with Liver Metastasis	1) Alan Melcher St James' Hosp Leeds, UK	Ongoing 6 patients	N/A	N/A
REO 014	2	IV	3 × 1010 (Fixed) (On Days 1 – 5, Q28 days)	Bone and soft tissue sarcoma	1) Monica Mita CTRC San Antonio, TX 2) Sanjay Goel Montefiore Bronx, NY 3) Scott Okuno Mayo Clinic Rochester, MN 4) Rashmi Chugh U of Michigan Ann Arbor, MI	Completed 53 patients (Mita et al., 2009)	No DLTs	19 pts SD for >2 mos 6 pts SD for > 6mos /44 (disease control rate CR+PR+SD = 43%)
REO 015 (with paclitaxel/ carboplatin)	2	IV	3 × 1010 (Fixed) (On Days 1 – 5 Q21 days)	Head and Neck Cancer	Monica Mita CTRC San Antonio, TX	Ongoing 7 pts	No DLTs to date	2 PR /6
REO 016 (with paclitaxel/ carboplatin)	2	IV	3 × 1010 (Fixed) (On Days 1 – 5 Q21 days)	NSCLC with K-ras and EGFR mutations	Miguel Villalona Ohio State Univ. Columbus, OH	Ongoing 6 patients	N/A	pending
REO 017 (with gemcitabine)	2	IV	3 × 1010 (Fixed) (On Days 2,4,9,11 Q21 days)	Pancreatic	Monica Mita CTRC San Antonio, TX	Pending	N/A	N/A
REO 018 (with paclitaxel/ carboplatin)	2-3	IV	3 × 1010 (Fixed) (On Days 1 – 5 Q21 days)	Head and Neck Cancer	TBD	Phase III ongoing	N/A	pending

2.4 Rationale for Reolysin® in Pancreatic Cancer

The prevalence of a constitutively active Ras pathway in pancreatic cancer and the specificity for Ras-transformed cells and relative non-pathogenic nature of reovirus present a strong rationale for Reolysin® as an anti-cancer therapy candidate for pancreatic cancer. A recent study that investigated the oncolytic effects of reovirus *in vitro* and examined the relationship between this susceptibility and an activated Ras signaling pathway, all five human pancreatic cancer cell lines tested were infectable with virus. Moreover, Ras activity in these cancer cell lines was elevated compared to that in the normal cell line, demonstrating that susceptibility to reovirus was associated with the Ras activity of these cells. In addition, reovirus was able to affect regression of xenograft tumors in immune-incompetent animal models [29].

2.5 The role of taxanes and platinums in pancreatic cancer:

Taxanes and platinums have been evaluated separately for patients with advanced pancreatic cancer. Single-agent docetaxel (ORR 5-15%, median OS 5.9-8.3 months) [30], weekly paclitaxel in the second- and third-line following gemcitabine failure (CR 5%, SD 27.7%, median OS 4.1 months) [31], and 24-hour infusional paclitaxel given every 3 weeks (ORR of 8% and median OS 5 months) [32] all have some modest activity for pancreatic cancer. Another study of 54 patients showed that the combination of protracted infusional 5-FU (an agent with single agent ORR 0-9%) with carboplatin in patients with advanced pancreas cancer yielded promising responses (ORR 17% with 2 CR, median OS 5.5 months) [33]. At the Ohio State University, we have recently completed a phase I study with the combination of carboplatin, paclitaxel, and capecitabine in patients with solid tumors with evidence of significant activity, most notably in pancreas cancer [34]. There were 11 patients with metastatic pancreas cancer enrolled on the study with 92% having failed at least one prior therapy (range 1-4) and > 95% of them failing prior gemcitabine. In this study, we observed 4 confirmed partial responses and 8 patients with prolonged stable disease with all patients showing evidence of a biochemical response.

2.6 Rationale for maximizing the effects of Reovirus with Carboplatin and Paclitaxel in pancreatic cancer

Numerous preclinical and clinical trials with Reolysin® have shown this agent to be well-tolerated and effective in a variety of solid malignancies. Moreover, these studies have demonstrated that Reolysin® had limited activity as a single agent, but that there was marked synergistic activity when Reolysin® was combined with chemotherapy such as carboplatin and paclitaxel. In non-small cell lung cancer (NSCLC), melanoma, and prostate cancer cell lines, the combination of paclitaxel and reovirus was invariably synergistic, even in cell lines such as the multidrug resistant NCI-H322M, with drug resistance to paclitaxel and/ or limited sensitivity to reovirus alone [26,35-37].

Given this preliminary data, it is very unlikely that Reolysin® will have any meaningful activity as a single agent in the treatment of pancreas cancer. However, Reolysin® in

combination with a paclitaxel-based therapy is an interesting and promising therapeutic approach. The apparent near-universal synergy, as well as, enhanced viral replication and oncolytic potential with immune modulation, demonstrated with the combination of paclitaxel and Reolysin®, endorses use of this combination, with the preferable addition of a platinum, to maximize the therapeutic effects of Reolysin®. Finally, the combination of carboplatin and paclitaxel may be a unique strategy for enhancing viral persistence in the blood by altering the ability of Reolysin® to evade immune recognition by "hitchhiking" within immune cells and subsequently travel to the tumor to provide oncolytic effects (adair RA et al. Sci Transl med, 2012).

Paclitaxel has been shown to enhance the virus-replication effect of reovirus, resulting in enhanced apoptotic rates compared to either agent alone. An NCI investigation of viral production rates in tumor cells demonstrated that paclitaxel**, <u>but not gemcitabine</u>**, could elicit enhanced viral production at 24 hours [26]. This enhanced virus-production effect of taxanes on reovirus has been independently confirmed [38] and appears to result in enhanced apoptotic rates compared to either agent alone [36,37].

The addition of chemotherapy also attenuates the neutralizing anti-reovirus antibody (NARA) response to reovirus administration, therefore enhancing efficacy and tumor response [27, 39]. An investigation of the patient samples from the UK phase I systemic monotherapy study demonstrated a robust increase of 250-fold in NARA [39] which peaked at day 14 and did not increase with subsequent challenge. Despite the induction of NARA, progressive reduction of tumor size as well as tumor markers after multiple cycles of Reolysin® have been reported in multiple Phase 1 and 2 studies, suggesting that immune antagonism does not entirely ablate tumor-targeting of the virus [15, 27]. It should be noted, however, that in animal models induction of NARA does impair efficacy and that attenuation of the NARA response in animal models has been demonstrated to enhance tumor response. Clinical trials investigating the combination of carboplatin and paclitaxel with Reolysin® have demonstrated a significantly attenuated and delayed NARA response. Typical increases in NARA lie between 27- and 81-fold for patients treated with carboplatin, paclitaxel and Reolysin®; compared to 250-fold for Reolysin® monotherapy [40]. This immune modulating effect is more robust with the combination of taxane and platinum agents, compared to taxanes alone [38]. Of note, when comparing results obtained in the carboplatin/paclitaxel combination study to the docetaxel combination study it was demonstrated that docetaxel alone provides little immune- dampening effect. This suggests that a taxane combination containing a platinum compound is superior to single taxane in terms of modulating the NARA response to Reolysin® (Oncolytics, personal communication). Based on these results and available pre-clinical data, it has been postulated that this immune modulation should enhance replication within the cancer cells, limit premature host clearance of the virus, and allow subsequent retargeting and delivery of virus to other malignant cells in the treated patients. Finally, even less is known regarding how chemotherapeutic agents when used in combination with Reolysin® will modulate viral persistence in the blood of treated patients. This trial allows for a unique opportunity to evaluate these clinically-relevant scientific questions.

Given the dismal outcome with gemcitabine-based therapy for patients with pancreatic cancer, it is imperative that we examine non-gemcitabine containing combination therapies in pancreas cancer. Reovirus, by targeting the Kras pathway, seems to be a rational therapeutic choice for the treatment of pancreatic cancer. There is a strong rationale for the combination of Reolysin®, carboplatin, and paclitaxel specifically in terms of overcoming drug resistance, enhancing the potency of the reovirus by augmenting replication, and suppressing induction of the neutralizing antibody response directed against the virus. There is also a strong rationale for the usage of platinums such as carboplatin and taxanes such as paclitaxel in patients with adenocarcinoma of the pancreas. We therefore propose this randomized phase II study to evaluate the activity of Reolysin®, carboplatin and paclitaxel compared to carboplatin and paclitaxel in the first-line treatment of patients with advanced pancreatic cancer. This study will allow patients in the non-Reolysin® arm to cross over to Reolysin® as described above.

2.7 Correlative Studies Background

The prevalence of a constitutively active Ras pathway in pancreatic cancer and the specificity of Reovirus Serotype 3-Dearing Strain to cause oncolysis in Ras-transformed cells form the basic rationale for using this agent for pancreatic cancer. The activation of MAP kinases ERK1 and ERK2 has been shown to be a good indicator of an activated Ras signaling pathway. In addition, there has been excellent correlation between the level of ERK-1/2 activity and susceptibility to reovirus infection [27]. We will therefore perform correlative studies to confirm an activated Ras pathway in pre-treatment tumor specimens.

In addition to tumor insensitivity and poor viral penetration into tumor, excessive viral clearance by the neutralizing immune response is a third potential barrier to effective Reolysin® therapy. In fact, synergy with combination chemotherapy may simply result from chemo-induced depletion of the neutralizing immune response. While the immune response can reduce active viral titer, it is unclear if this response inhibits tumor cell destruction or enhances it by directing phagocytes and other immune effector cells to virus-infected tumor cells through cytokine signaling in the tumor microenvironment. Indeed, infection with reovirus also can induce potent immunomodulatory properties that may potentially contribute to tumor eradication For example, tumor infection by reovirus can upregulate the activation and cytotoxic properties of natural killer (NK) cells and $CD8^+$ T cells (Prestwich, J Immunol., 2009). Finally, the effects of carboplatin and paclitaxel on the ability of reovirus to persist and evade immune recognition in the peripheral blood has not been explored in any published study to date. These data suggest that reovirus may also regulate innate and adaptive immune responses or viral persistence to facilitate tumor clearance. We propose to study how co-administration of chemotherapy with carboplatin plus paclitaxel will modulate the immune response to Reolysin® and promote an anti-tumor effect. We believe that modulating the immune response to Reolysin® may lead to lower levels of NARA and altered tumor sensitivity to Reolysin® infection and/or replication through modulation of cytokine and immune effector cell phenotype. Similarly, the persistence of Reolysin® will be evaluated in patients treated with this combination regimen to determine whether the process of viral

hitchhiking is relevant to therapeutic effects. Therefore in the proposed panel of correlative studies we will determine how combined treatment with reovirus and chemotherapy modulates NARA titers, the inflammatory cytokine profile, immune effector cell phenotype function and viral persistence.

Chemotherapy could alter immunologically relevant features of the tumor cell (e.g. antigen expression) at the time in which Reolysin®-activated immune effectors migrate to the tumor site [41]. This could potentially alter the general cellular phenotype, antigen presentation, or activation of cytotoxic lymphocytes. In addition, chemotherapy with carboplatin and paclitaxel has been suggested to augment the anti-tumor response via the induction of a temporary reconstitution of cytotoxic immune effector cells [42]. The present trial will also provide a unique opportunity to test whether the presence of Reolysin® in combination with carboplatin plus paclitaxel might modulate the immune system and potentially the response to therapy.

Suppressed immune function associated with advanced malignancy is mediated in part via T regulatory cells (T reg) and myeloid derived suppressor cells (MDSC). These cell populations are markedly elevated in both murine and human tumor models and can serve as a barrier to immune mediated recognition of pancreatic cancer through numerous mechanisms [43]. Furthermore, neutralizing these cells or their suppressive properties has been shown to adversely impact the tumor [43]. Prior studies have shown a significant decrease in the level of circulating T regs two weeks following a single infusion of carboplatin and paclitaxel in patients with ovarian cancer [42]. To our knowledge, there are no published studies that have evaluated the effect of reovirus infection on the level of T reg or MDSC in patients with advanced malignancies. Due to the potential for broad cytotoxic capacity of carboplatin plus paclitaxel, it is likely that treatment with these chemotherapeutic agents will lead to at least a transient decrease in the systemic level of these suppressive cell populations. Therefore, the present study will provide a unique and important opportunity to determine whether modulation of immune suppressor cell subsets represents an additional mechanism by which reovirus can elicit anti-tumor responses. It is possible that a portion of anti-tumor activity from this treatment regimen could be mediated by a reduced systemic level of T reg and MDSC. An alternative possibility is that the elevated systemic levels of immune suppressive cells could provide an inherent advantage for viral persistence in a tumor-bearing host. Therefore, we hypothesize that this state of elevated immune suppressor cells in cancer patients can be manipulated to provide an advantage for the maximal oncolytic effect of Reolysin.

3 PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically confirmed adenocarcinoma of the pancreas that is recurrent or metastatic. Cytological confirmation is not allowed on this study, as tissue is needed for correlative science analysis. Paraffin embedded tissue from tumor blocks will be required from patients before enrolling on this study. Diagnosis of pancreas cancer with histologic confirmation of adenocarcinoma would suffice.

3.1.2 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension per RECIST 1.1 (longest diameter to be recorded) as ≥10 mm with spiral CT scan (CT scan slice thickness no greater than 5 mm) Malignant lymph nodes will be considered measurable if they are ≥15 mm in short axis. See Section 11 for the evaluation of measurable disease. For patients previously irradiated, the measurable lesion must be outside the radiated field.

3.1.3 Patients must not have received any prior chemotherapy in metastatic setting. Patients who have received prior chemotherapy in the adjuvant setting will not be eligible for our study. Patients should not have received prior Reolysin. Prior palliative radiation therapy or major surgery must have occurred at least 28 days prior to study enrollment. Prior minor surgeries (such as laparoscopies) must have occurred at least 14 days prior to study enrollment. Prior minor procedures such as biopsies and mediport placement must have occurred at least 48 hours prior to study enrollment.

3.1.4 Age ≥18 years.

3.1.5 ECOG performance status ≤1 (Karnofsky ≥70%, see Appendix A).

3.1.6 Patients must have normal organ and marrow function as defined below:

- Absolute neutrophil count ≥ 1.5 x 10^9/L SI units
- Platelets ≥ 100 x$10^{9/}$L SI units
- Hemoglobin ≥ 8.5 g/dL (gm/L) SI units
- Serum creatinine ≤ 1.5, or Creatinine clearance ≥ 60 ml/min (calculated using the Cockcroft-Gault equation).
- Bilirubin ≤ ULN (≤ 2x ULN if it is non-rising for a period of 10 days prior to initiation of therapy)
- AST/ALT ≤ 3 x ULN
- Troponin I < ULN

3.1.7 All patients must have signed an informed consent indicating that they are aware of the neoplastic nature of their disease and have been informed of the procedures of the protocol, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts.

3.1.8 The effects of Reolysin® on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because the other chemotherapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Patients must be able to avoid direct contact with pregnant or nursing women, infants and immunocompromised individuals while on study and for ≥3 weeks following the last dose of Reolysin administration.

3.1.9 All patients must be willing and able to comply with scheduled visits, the treatment plan, and laboratory tests.

3.2 Exclusion Criteria

3.2.1 Patients may not be receiving any other investigational agents or concurrent therapy with other anti-cancer agents while on study.

3.2.2 Patients with untreated brain metastases will be excluded from this clinical trial. However, patients with resected oligometastasis are eligible if postresection MRI demonstrates resolution. Gamma-knife treated patients are also eligible if there are no more than two treated metastases confined to the same area of the brain and a post treatment MRI shows a decrease in the metastases.

3.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Reolysin® or other agents used in the study.

3.2.4 Patients may not have received any viral-based therapy within the past 6 months.

3.2.5 Patients must have NO continuing acute toxic effects (except alopecia) of any prior radiotherapy, chemotherapy, or surgical procedures. All such effects must have resolved to Common Terminology Criteria for Adverse Events (CTCAE, v. 4) Grade ≤ 1 prior to study enrollment.

3.2.6 Patients must not have grade 2 or higher baseline peripheral neuropathy, according to CTCAE v. 4.

3.2.7 Patients with uncontrolled cardiac dysfunction or arrhythmia, including a myocardial infarction in the preceding 6 months, known cardiac ejection fraction < 40%, symptomatic congestive heart failure, or unstable angina pectoris.

3.2.8 Patients must not be receiving current systemic immunosuppressive therapy.

3.2.9 Patients must not have known HIV infection or active hepatitis B or C.

3.2.10 Patients must not have uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, or known psychiatric illness/ social situations that would limit compliance with study requirements.

3.2.11 Patients must not have dementia or altered mental status that would prohibit informed consent.

3.2.12 Patients must not have any other known severe, acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or study drug administration or may interfere with the interpretation of study results and, in the judgment of the Principal Investigator, would make the patient inappropriate for this study.

3.2.13 Pregnant women are excluded from this study because of the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Reolysin®, breastfeeding should be discontinued while the mother is being treated with the agents in this clinical trial.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial. Pancreatic cancer is primarily a disease of adults and its biology and pathogenesis may be different in children; therefore, children < 18 years of age will be excluded.

4 REGISTRATION PROCEDURES

4.1 General Guidelines

Eligible patients will be entered on study centrally at the Ohio State University by the Study Coordinator. All sites should call the Subsite Study Coordinator, *Jennifer Sexton at (614) 366-5642* to verify patient slot availability. The required forms the Eligibility Checklist and Registration Form can be found in Appendix D and E.

Following registration, patients should begin protocol treatment within 5 working days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Subsite Study Coordinator should be notified of cancellations as soon as possible.

Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only

after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) except for Group studies.

4.2 Registration Process

To register a patient, the following documents should be completed by the research nurse or data manager and faxed to *Jennifer Sexton* at (614) 366-5642:

- Copy of required laboratory tests
- Signed patient consent form
- HIPAA authorization form
- Eligibility Checklist (Appendix D)
- Registration Form (Appendix E)
- Additional source documents pertaining to verification of eligibility.

The research nurse or data manager at the participating site will then call the Subsite Study Coordinator, *Jennifer Sexton at telephone: 614-366-5642* to verify eligibility. To complete the registration process, the Coordinator will

- assign a patient study number
- register the patient on the study
- fax or e-mail the patient study number and dose to the participating site
- call the research nurse or data manager at the participating site and verbally confirm registration.

5 TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks Reolysin®, carboplatin, paclitaxel are described in Section 7. Appropriate dose modifications Reolysin®, carboplatin, paclitaxel are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

5.1.1 Arm A (and Arm B if Reolysin®) given upon progression): Reolysin®, Carboplatin, and Paclitaxel

5.1.1.1 Premedication:

All patients should be premedicated prior to paclitaxel administration in order to prevent severe hypersensitivity reactions. According to the package insert, the recommended premedication consists of dexamethasone 20 mg orally administered approximately 12 and 6 hours before paclitaxel; diphenhydramine 50 mg IV or PO 30 to 60 minutes prior to paclitaxel; and cimetidine (300 mg) or ranitidine (50 mg) or famotidine (20 mg) IV or PO 30 to 60 minutes prior to paclitaxel. However, pre-

medication regimen for paclitaxel may be administered according to institutional guidelines.

5.1.1.2 **Administration:**

Patients will receive paclitaxel intravenously as a 3-hour infusion at a dose of 175 mg/m^2, followed by carboplatin intravenously as a 30-minute infusion at a dose AUC 5 mg/mL·minute, both on Day 1 of each 21-day cycle. For dosing of carboplatin, creatinine clearance will be calculated using the Cockcroft-Gault equation and total dose will be calculated using the Calvert formula (See Figure 1). Of note, the estimated GFR will be capped at 125 ml/min for all patients. For patients who have a calculated GFR greater than 125 ml/min, use "125" as their GFR to calculate their Carboplatin dose. REOLYSIN® will be administered after paclitaxel and carboplatin as a 60-minute intravenous infusion at a dose of $3x10^{10}$ $TCID_{50}$ / day, on Days 1-5 of each cycle. On Day 1, it will be given following completion of the carboplatin infusion. All three agents may be administered via peripherally or centrally inserted venous catheters.

The following medications (or equivalent) should be available at the bedside for use in case of an immune-mediated reaction: epinephrine (0.5-1 mL subcutaneous injection 1:1000) and diphenhydramine HCl 50 mg for intravenous injection.

When discharged, the patient and the family will receive an outpatient information sheet regarding infectious precautions that they should follow both at home and in public [See Appendix B]. Patients should avoid taking acetaminophen with Reolysin®. Whenever suitable, physicians should utilize alternative medications, such as ibuprofen or aspirin. Recent preclinical and clinical information has indicated a possible interaction between Reolysin and acetaminophen (paracetamol, APAP) causing possible hepatotoxicity resulting in increases in liver enzymes (ALT/SGPT and GGT). (Haller et al., 1995, J Virol 69:357:364; Maddox et al., 2010, J. Toxicol Environ Health A. 73:58-73).

5.1.2 **Arm B : Carboplatin and paclitaxel**

5.1.2.1 **Premedication:**

All patients should be premedicated prior to paclitaxel administration in order to prevent severe hypersensitivity reactions. According to the package insert, the recommended premedication consists of dexamethasone 20 mg orally administered approximately 12 and 6 hours before paclitaxel; diphenhydramine 50 mg IV or PO 30 to 60 minutes prior to paclitaxel; and cimetidine (300 mg) or ranitidine (50 mg) or famotidine (20 mg) IV or PO 30 to 60 minutes prior to paclitaxel. However, pre-medication regimen for paclitaxel may be administered according to institutional guidelines.

5.1.2.2 **Administration:**

Patients will receive paclitaxel intravenously as a 3-hour infusion at a dose of 175 mg/m^2, followed by carboplatin intravenously as a 30-minute infusion at a dose AUC 5 mg/mL·minute, both on Day 1 of each 21-day cycle. For dosing of carboplatin, creatinine clearance will be calculated using the Cockcroft-Gault equation and total dose will be calculated using the Calvert formula (See Figure 1). Of note, the estimated GFR will be capped at 125 ml/min for all patients. For patients who have a calculated GFR greater than 125 ml/min, use "125" as their GFR to calculate their Carboplatin dose. Both agents may be administered via peripherally or centrally inserted venous catheters.

5.2 General Concomitant Medication and Supportive Care Guidelines

Flu-like symptoms (fever, headache, myalgias, arthralgias) may be treated with aspirin, ibuprofen or antihistamines as clinically indicated per the investigators' judgment. Recent preclinical and clinical information has indicated a possible interaction between Reolysin® and acetaminophen (paracetamol, APAP) causing possible hepatotoxicity resulting increases in liver enzymes ALT/SGPT and GGT. Patients should avoid taking acetaminophen with Reolysin®.

Diarrhea may be treated initially with loperamide (Imodium®) as clinically indicated, with additional interventions per the investigators' judgment.

Nausea and vomiting may be treated with $5HT_3$ receptor antagonists as clinically indicated, with additional interventions per the investigators' judgment.

Hematologic support with granulocyte-colony stimulating factor and/or transfusions of packed red blood cells and platelets may be given as clinically indicated, per ASCO, NCCN or institutional guidelines. The use of erythropoietin will be in strict compliance with FDA recommendations in the current prescribing information.

All **concomitant medications** must be recorded in the patient's Case Report Form (CRF). No surgery (except biopsy), radiation, investigational treatment, immunotherapy or additional chemotherapy is allowed during the course of this study. Concurrent bisphosphonates are allowed. Anticoagulant therapy is also permitted. Apart from the excluded concomitant therapies listed above, other drugs can be administered at the discretion of the investigator. There is no expected cytochrome P450 isoenzyme drug-drug interactions with the agents used in this study, except for paclitaxel. CYP450 2C8 and 3A4 inducers and inhibitors may affect paclitaxel metabolism (please refer to package insert for more information).

5.3 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression**,

- Severe intercurrent illness that prevents further administration of treatment,
- The following adverse event(s)*,
  - ≥ grade 3 allergic reaction
  - ≥ grade 3 cardiac or neurological toxicity
  - ≥ grade 3 non-hematologic toxicity that does not resolve to ≤ grade 1 within 3 weeks
  - decrease of LVEF (left ventricular ejection fraction) to < 50% or decrease of LVEF by ≥ 10% from baseline.
- Severe adverse events that warrant more than two dose reductions
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

The Case Report Form should contain a precise description of any discontinuation or withdrawal from the protocol treatment and a conclusion as to the reason for the discontinuation

* Note that patients in Arm A who experience hematologic or non-hematologic toxicity that requires more than 2 dose reductions may continue treatment with Reolysin® as monotherapy (if toxicity is deemed to be related specifically to the carboplatin and paclitaxel). Patients who continue treatment with only Reolysin® will be followed as before and the same criteria above hold for determining when they should discontinue treatment.

** Patients in Arm B who have documented disease progression will be allowed to receive Reolysin® in addition to carboplatin and paclitaxel. However, any follow-up assessment after their initial progression that leads to the addition of Reolysin® to their treatment regimen will not be included in assessing the primary endpoint for this trial. In the event that a patient is crossed-over to receive Reolysin, the treatment will begin from cycle 1 day 1. Once patients are treated with this new therapeutic combination, the same criteria described above will hold for determining when patients should discontinue treatment.

5.4 Duration of Follow Up

Patients will have a follow-up visit one month following the end of study treatment and every two months after discontinuation of study treatment or until death, whichever occurs first, in order to determine date of progression and survival outcome. Patients removed from study for unacceptable adverse events considered as possibly related, probably related or definitely related to the protocol treatment will be followed until the adverse event is resolved, stabilized or viewed as permanent.

5.5 Criteria for removal from Study

Patients will be removed from study in terms of their follow-up per protocol when any of the criteria (listed below) applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

- Death
- Intercurrent illness that prevents further administration of treatment
- Administration of alternate anti-cancer therapy
- Withdrawal of consent
- Protocol violation
- Lost to follow-up

6 DOSING DELAYS/DOSE MODIFICATIONS

There will be no dose reductions for Grade 2, 3, or 4 nausea or vomiting that was suboptimally treated with appropriate antiemetics. There will be no dose reduction for grade 2 alopecia. There will be no dose reductions for any grade lymphopenia or Grade 1, 2 or 3 anemia. If toxicity is deemed to be related to carboplatin and/ or paclitaxel rather than Reolysin®, then treatment may continue with single agent Reolysin®

NOTE: If toxicities are related to Taxol and Taxol has to be discontinued, treatment with Carboplatin will be continued.

In the event of a Holiday, change in clinic or chemotherapy date or planned vacation, social hardship or at the patient request, the start of a new cycle may be delayed up to 2 weeks and at the investigator's discretion. This should not be done on a routine basis.

6.1 Arms A and B: Carboplatin and Paclitaxel with or without Reolysin®,

NOTE: ANC and platelet levels must be grade ≤ 1 for retreatment.

All treatment related toxicities must have recovered to Grade ≤ 1 (or tolerable grade 2 or baseline) for re-treatment. Dose delays beyond 3 weeks will require removal from the study.

For toxicities occurring and resolving prior to the day of treatment, dose modifications will be carried out for the following:

- ANC < 0.5 x 10^9/L lasting for > 7 days or ANC < 0.5 x 10^9/L with fever (>100.5° F) lasting >48 hours.
- Platelet count < 25 x 10^9/L.
- Any other drug-related, non-hematologic grade 3/4 toxicity, except alopecia,
- If dose changes more than 5% of previous dose, follow institutional guidelines for dose adjustment.

Dose modification for hematologic and non-hematologic toxicity possibly, probably, or definitely related to carboplatin and/ or paclitaxel:

Toxicity and Grade	Carboplatin	Paclitaxel
ANC < 0.5 x 10^9/L lasting for > 7 days or ANC < 0.5 x 10^9/L with fever (>100.5° F) lasting >48 hours.	1st occurrence = AUC 4 2nd occurrence = AUC 3 3rd occurrence = Discontinue	1st occurrence = 75% of original dose 2nd occurrence = 50% of original dose 3rd occurrence = Discontinue
Platelet count < 25 x 10^9/L.	1st occurrence = AUC 4 2nd occurrence = AUC 3 3rd occurrence = Discontinue	1st occurrence = 75% of original dose 2nd occurrence = 50% of original dose 3rd occurrence = Discontinue
Any other drug-related, non-hematologic grade 3/4 toxicity, except alopecia.	1st occurrence = AUC 4 2nd occurrence = AUC 3 3rd occurrence = Discontinue	1st occurrence = 75% of original dose 2nd occurrence = 50% of original dose 3rd occurrence = Discontinue
For Neurotoxicity and Cardiotoxicity	For grade 3 neurotoxicity or cardiotoxicity or worse, carboplatin will be discontinued.	Reduce by 25% for grade 2 neurotoxicity or cardiotoxicity and discontinue if there is a grade 3 or worse peripheral neuropathy or cardiotoxicity.

Dose reduction for diarrhea related to Reolysin:

For grade 3 or 4 diarrhea considered as possibly related, probably related or definitely related to REOLYSIN®, all remaining daily doses of REOLYSIN® will be held for that treatment cycle and the daily dose of REOLYSIN® for all subsequent cycles will be decreased to 1 x 10^{10} $TCID_{50}$. If severe diarrhea recurs after dose reduction of REOLYSIN® and despite optimal supportive therapy, the patient will be taken off study.

Dose reductions for other toxicities related to Reolysin[a]:

CTCAE Category	Adverse Event	Dose Modification
Based on Interval Adverse Event		
Blood/Bone marrow	Grade 4	Hold any remaining doses of Reolysin and decrease dose to 1 x 10^{10} $TCID_{50}$ for all subsequent cycles.
All Non-hematologic categories	≥ Grade 3[b]	Hold any remaining doses of Reolysin and decrease dose to 1 x 10^{10} $TCID_{50}$ for all subsequent cycles.
Cardiac toxicity	≥ Grade 2	Hold any remaining doses of Reolysin and decrease dose to 1 x 10^{10} $TCID_{50}$ for all subsequent cycles.
At Scheduled Retreatment (Day 1 of each cycle)		
Blood/ Bone Marrow	≥ Grade 3	Hold treatment until toxicity is ≤ Grade 1, then retreat as per interim toxicity. If no resolution within 4 weeks, patient should permanently discontinue study treatment.
Non-hematologic	≥ Grade 3	Hold treatment until toxicity is ≤ Grade 1,

categories		then retreat as per interim toxicity. If no resolution within 4 weeks, patient should permanently discontinue study treatment.
Cardiac toxicity[c] (except left ventricular diastolic dysfunction (LVDD))	Grade 2	Hold treatment until toxicity is ≤ Grade 1, then retreat as per interim toxicity. If no resolution within 4 weeks, patient should permanently discontinue study treatment.
Heart Failure	Grade 2	Discontinue treatment and permanently remove patient from the study.
Cardiac toxicity	≥ Grade 3	Discontinue treatment and permanently remove patient from the study.
Allergic reaction	≥ Grade 3	Discontinue treatment and permanently remove patient from the study.

a- These dose modifications will apply only to toxicities which are felt to be possibly, probably, or definitely related to Reolysin by AE attribution.

b- **Exceptions to the above for dose modification will include the following signs and symptoms:**
- Grade 3 flu-like signs and/or symptoms, i.e., chills/rigors, headache, fatigue, dizziness, cough, sore throat, arthralgia, and/or myalgia of ≤72 hours duration.
- Grade 3 fever of ≤72 hours duration.

Note: The duration limitation of 72 hours will apply when the signs or symptoms listed above are **continuous** over a 72 hour period.

c- Patients should also discontinue treatment and be permanently removed from the study if LVEF (left ventricular ejection fracture) decreases ≥ 10% from baseline.

7 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited (via CTEP-AERS) reporting **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

7.1.1 Adverse Event List(s) for Reolysin®:

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Reolysin® (NSC 729968)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 528 patients.* Below is the CAEPR for Reolysin®.

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.2, April 19, 2013[1]

Adverse Events with Possible Relationship to Reolysin® (CTCAE 4.0 Term) [n= 528]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)** (formerly known as ASAEL)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Diarrhea		***Diarrhea (Gr 2)***
	Nausea		***Nausea (Gr 2)***
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		***Chills (Gr 2)***
	Fatigue		***Fatigue (Gr 2)***
	Fever		***Fever (Gr 2)***
	Flu like symptoms		***Flu like symptoms (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased[2]		***Alanine aminotransferase increased (Gr 2)***
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 2)***
	Neutrophil count decreased		***Neutrophil count decreased (Gr 2)***
	Platelet count decreased		***Platelet count decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 2)***
	Myalgia		***Myalgia (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Headache		***Headache (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pharyngolaryngeal pain		***Pharyngolaryngeal pain (Gr 2)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Recent preclinical and clinical information has indicated a possible interaction between Reolysin® and acetaminophen (paracetamol, APAP) causing possible hepatotoxicity, resulting in increases in liver enzymes ALT/SGPT and GGT. Patients should avoid taking acetaminophen with Reolysin®. Whenever suitable, physicians should utilize alternative medications, such as ibuprofen or aspirin.

[3]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Also reported on Reolysin® trials but with the relationship to Reolysin® still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Febrile neutropenia
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Cardiac disorders - Other (CK-MB); Left ventricular systolic dysfunction; Supraventricular tachycardia
EAR AND LABYRINTH DISORDERS - Vertigo
EYE DISORDERS - Eye disorders - Other (optic neuritis); Eye pain
GASTROINTESTINAL DISORDERS - Abdominal pain; Constipation; Dyspepsia; Rectal pain
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Gait disturbance; Injection site reaction; Malaise; Pain
INFECTIONS AND INFESTATIONS – Infection[3]
INVESTIGATIONS - Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Cardiac troponin I increased; Creatinine increased; Ejection fraction decreased; GGT increased[2]; Investigations - Other (cerebrospinal fluid WBC increased); Investigations - Other (LDH); Lipase increased; Serum amylase increased; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperuricemia; Hypoalbuminemia; Hyponatremia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Chest wall pain; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (musculoskeletal stiffness); Neck pain
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (tumor bleeding)
NERVOUS SYSTEM DISORDERS - Ataxia; Dizziness; Dysgeusia; Dysphasia; Ischemia cerebrovascular; Seizure; Somnolence; Syncope
PSYCHIATRIC DISORDERS - Confusion; Insomnia
RENAL AND URINARY DISORDERS - Renal and urinary disorders - Other (bilateral nephrosis); Urinary frequency
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Bronchopulmonary hemorrhage; Bronchospasm; Cough; Dyspnea; Pharyngeal mucositis; Respiratory failure
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Hyperhidrosis; Pruritus; Rash maculo-papular
VASCULAR DISORDERS - Hypertension; Hypotension; Thromboembolic event; Vascular disorders - Other (bleeding from the left inguinal node)

Note: Reolysin® in combination with other agents could cause an exacerbation of any adverse event

currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.1.2 **Adverse Event List(s) for Commercial Agents:**

7.1.2.1 Carboplatin [44]:

Adverse event	Intravenous Administration N=51 (%)			
	All	*At least Possibly Related to Rx*	*Grade ≥ 3*	*Both Possibly Related & ≥ Grade 3*
Blood and Lymphatic System Disorders				
Anemia	10 (20)	1 (2)	3 (6)	0
Lymphopenia	6 (12)	4 (8)	5 (10)	2(4)
Neutropenia	5 (10)	5 (10)	3 (6)	2 (4)
Ear/Eye Disorders				
Gastrointestinal Disorders				
Abdominal Pain	15 (29)	2 (4)	0	0
Constipation	12 (24)	1 (2)	0	0
Diarrhea	13 (25)	4 (8)	0	0
Nausea	14 (27)	7 (14)	0	0
Vomiting	6 (12)	3 (6)	0	0
General Disorders				
Chills	8 (16)	7 (14)	0	0
Fatigue	26 (51)	14 (27)	3 (6)	1 (2)
Influenza-Like Illness	10 (20)	10 (20)	1 (2)	1(2)
Pain	2 (4)	1 (2)	0	0
Fever	31 (61)	27 (53)	1 (2)	0
Infections				
Urin.Tract Infect.	9 (18)	0	0	0
Investigations				
Incr. ALT	5 (10)	0	1 (2)	0
Incr.AST	5 (10)	0	1 (2)	0
Metabolic /Nutrition Disorders				
Anorexia	14 (27)	6 (12)	0	0
Musculoskeletal/Connective Tissue Disorders				
Arthralgia	8 (16)	4 (8)	1 (2)	0
Myalgia	6 (12)	5 (10)	0	0
Back Pain	5 (10)	2 (4)	1 (2)	0
Pain in Extremity	5 (10)	0	0	0
Nervous/Psychiatric System Disorders				
Dizziness	7 (14)	3 (6)	0	0
Headache	21 (41)	17 (33)	1 (2)	0
Renal/Urinary Disorders				
Dysuria	5 (10)	1 (2)	0	0
Respiratory Disorders				
Dyspnoea	10 (20)	0	4 (8)	0
Pharyng.Lar.Pain	7 (14)	5 (10)	0	0

7.1.2.2 **Paclitaxel [45]:**

		Percent of Patients (n=812)
• ***Bone Marrow***		
—Neutropenia	<2000/mm^3	90
	<500/mm^3	52
—Leukopenia	<4000/mm^3	90
	<1000/mm^3	17
—Thrombocytopenia	<100,000/mm^3	20
	<50,000/mm^3	7
—Anemia	<11 g/dL	78
	<8 g/dL	16
—Infections		30
—Bleeding		14
—Red Cell Transfusions		25
—Platelet Transfusions		2
• ***Hypersensitivity Reaction***[b]		
—All		41
—Severe[†]		2
• ***Cardiovascular***		
—Vital Sign Changes[c]		
—Bradycardia (n=537)		3
—Hypotension (n=532)		12
—Significant Cardiovascular Events		1
• ***Abnormal ECG***		
—All Pts		23
—Pts with normal baseline (n=559)		14
• ***Peripheral Neuropathy***		
—Any symptoms		60
—Severe symptoms[†]		3
• ***Myalgia/Arthralgia***		
—Any symptoms		60
—Severe symptoms[†]		8
• ***Gastrointestinal***		
—Nausea and vomiting		52
—Diarrhea		38
—Mucositis		31
• ***Alopecia***		87
• ***Hepatic*** (Pts with normal baseline and on study data)		
—Bilirubin elevations (n=765)		7
—Alkaline phosphatase elevations (n=575)		22
—AST (SGOT) elevations (n=591)		19
• ***Injection Site Reaction***		13

[a] Based on worst course analysis.
[b] All patients received premedication.
[c] During the first 3 hours of infusion.
[†] Severe events are defined as at least Grade III toxicity.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade: CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section 7.1 above) for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR (Section 7.1.1).

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP-Adverse Event Reporting System), accessed via the CTEP home page (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" which can be downloaded from the CTEP home page (http://ctep.cancer.gov). These requirements are briefly outlined in the table below (**Phase 2 and 3 Trials**).

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients. Subsite institutions must ensure that the CTEP-AERS report and supporting documentation is sent to the Subsite Study Coordinator.

7.3.3 Expedited Reporting Guidelines – CTEP-AERS Reporting Requirements for Adverse Events that occur within 30 Days[1] of the Last Dose of the Investigational Agent on Phase 2 and 3 Trials

Phase 2 and 3 Trials									
	Grade 1	**Grade 2**	**Grade 2**	**Grade 3**		**Grade 3**		**Grades 4 & 5[2]**	**Grades 4 & 5[2]**
				Unexpected		**Expected**			
	Unexpected and Expected	**Unex-pected**	**Expected**	**with Hospitali-zation**	**without Hospitali-zation**	**with Hospitali-zation**	**without Hospitali-zation**	**Unex-pected**	**Expected**
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur <u>greater</u> than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**
CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:
- Grade 4 and Grade 5 unexpected events

CTEP-AERS 10 calendar day report:
- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although an CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

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Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

- Expedited AE reporting timelines defined:
  - "24 hours; 5 calendar days" – The investigator must initially report the AE via CTEP-AERS within <u>24 hours</u> of learning of the event followed by a complete CTEP-AERS report within <u>5 calendar days</u> of the initial 24-hour report.
  - "10 calendar days" - A complete CTEP-AERS report on the AE must be submitted within <u>10 calendar days</u> of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must <u>also</u> be reported in routine study data submissions.**

7.5 Data Safety Monitoring Plan

The data and safety monitoring plan will involve the continuous evaluation of safety, data quality and data timeliness. Investigators will conduct continuous review of data and patient safety at their regular Disease Group meetings (at least monthly) and the discussion will be documented in the minutes. The PI of the trial will review toxicities and responses of the trial where applicable at these disease center meetings and determine if the risk/benefit ratio of the trial changes. Frequency and severity of adverse events will be reviewed by the PI and compared to what is known about the agent/device from other sources; including published literature, scientific meetings and discussions with the sponsors, to determine if the trial should be terminated before completion. Serious adverse events and responses will also be reviewed by the OSUCCC Data and Safety Monitoring Committee (DSMC). The PI will also submit a progress report (biannually for Phase II and quarterly for Phase I) that will be reviewed by the committee per the DSMC plan. All reportable Serious Adverse Events (SAE) will also be reported to the IRB of record as per the policies of the IRB.

8 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 7.1.

8.1 CTEP-Supplied Investigational Agents

8.1.1 Reovirus Serotype 3 – Dearing Strain (NSC # 729968)

Other Names: Reovirus Serotype 3 – Dearing Strain

Classification: Cell-selective replication-competent oncolytic virus

Description: Reovirus (Respiratory Enteric Orphan virus) is a live, naturally occurring, ubiquitous, non-enveloped human virus of the Reoviridae family, with a genome consisting of 10 double-stranded RNA segments. Serotype 3 – Dearing Strain is one of three distinct reovirus serotypes.

Mechanism of Action: Reovirus selectively replicates in, and is cytopathic to, Ras-activated cells, while sparing normal cells. Preferential replication in and lysis of Ras-activated cells appears to be due to inhibition of the antiviral effects of double-stranded RNA-activated protein kinase (PKR) in these cells. In normal non-Ras-activated cells, PKR autophosphorylates in the presence of viral transcripts, becomes activated, and inhibits viral protein synthesis, preventing viral replication. Ras activated cells inhibit the autophosphorylation of PKR, keeping it in an inactive state, allowing viral translation and cellular infection to occur and eventually, oncolysis to take place.

How supplied: REOLYSIN® is provided by Oncolytics Biotech Inc. and distributed by the Pharmaceutical Management Branch, CTEP, DCTD, NCI as a clear to translucent to opaque, colorless to light blue to white aqueous liquid supplied in Type I glass vials containing 1 mL of Reovirus Serotype 3 – Dearing Strain.
Note: The viral titer may vary between lots, requiring changes to dose preparation instructions. **Use extreme caution when preparing each dose.**

REOLYSIN® is supplied in vials containing 1 mL of the virus at a final viral concentration titer of 4.5×10^{10} $TCID_{50}$/mL formulated in phosphate-buffered saline containing 3% Mannitol, 2% Histidine, 2% Sorbitol, 0.01% Polysorbate 80 and 2mM $MgCl_2$.

Preparation: Thaw vials completely at room or refrigerated temperature. Thaw time at room temperature is approximately 10 minutes. Prepare doses immediately upon thawing. Perform all dilutions of the virus with 0.9% sodium chloride for injection, USP. **Note the concentration of the current supply of REOLYSIN® on your institutional preparation guidelines to avoid potentially serious dosing errors.** Withdraw and inject the measured dosage volume of REOLYSIN® into a PVC or non-DEHP containing IV bag (e.g., IV bags composed of polyethylene, polypropylene, or polyolefin) containing 250 mL 0.9% sodium chloride. DO NOT use IV bags composed of ethylene-vinyl acetate (EVA). Mix by GENTLY inverting the bag several times.

Storage: Store intact vials of REOLYSIN® at -20º C or colder

Stability: Shelf-life stability studies of the intact vials are ongoing. Do not re-freeze thawed vials. All vials are intended for single-use only and do not contain a preservative; discard unused agent within 8 hours after initial entry of the vial. Store prepared doses of REOLYSIN® at refrigerated temperature. Administer within 8 hours of dilution in 0.9% sodium chloride if prepared in a non-DEHP-containing IV bag or administer within 6 hours of dilution in 0.9% sodium chloride if prepared in a PVC IV bag.

Routes of Administration: Intravenously as per protocol.

Method of Administration: Administer REOLYSIN® as a 60-minute intravenous infusion using a PVC administration set. A volumetric pump may be used to control the administration rate.

Special Handling: Handle Reolysin® as an infectious biohazardous agent, using universal precautions for infectious materials and Biosafety Level 2 guidelines. Consider all materials that have been in contact with the virus as infectious biohazards for disposal in accordance with institutional biosafety policy and procedures for infectious biohazardous waste management. Incinerate waste materials according to institutional policies and local, state, and federal regulations for infectious biohazardous waste.

Participating clinical trial sites should consult with their local Institutional Biosafety Committee (IBC) for site-specific recommendations regarding preparation,

handling and disposal; healthcare worker precautions; and patient care implications:

General Preparation, Handling and Disposal Recommendations
For more information about biohazard risk group classification and biohazard safety levels see Biosafety in Microbiological and Biomedical Laboratories; 5th Edition, February, 2007. U. S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health (http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm).

1. Prepare a biosafety manual which advises personnel of special hazards and specific practices and procedures.

2. Allow only personnel knowledgeable of potential hazards and meeting specific entry requirements in preparation or storage areas.

3. Strictly adhere to standard microbiological practices and techniques and use appropriate infection control measures (e.g., thorough hand washing) after handling any materials.

4. Restrict access to preparation areas while dose preparation is in progress.

5. Do not eat, drink, handle contact lenses or apply cosmetics in the work area. Institute and follow policies for safe sharps handling.

6. Use only needle-lock syringes and needles for dose preparation. Use extreme caution to prevent auto-inoculation. Do not bend, shear, or replace the needle guard from the syringe by hand following use. Promptly place used needles and syringes in puncture-resistant biohazard containers for appropriate disposal.

7. Perform all dose preparations in a certified Class II biological safety cabinet. Avoid direct contact or inhalation, and use eye protection, mask, gloves, and gown at all times during handling. Wash hands thoroughly after removing gloves.

8. Perform all procedures carefully to minimize aerosol creation.

9. Decontaminate all equipment and work surfaces with an appropriate disinfectant (minimum of 2% bleach solution for this agent; or in accordance with institutional biosafety policies and procedures) prior to and after dose preparation, and especially after overt spills, splashes, or other contamination.

10. Prior to dose preparation, decontaminate the biological safety cabinet with sterile gauze soaked in a minimum of 2% bleach solution, or other appropriate disinfectant suitable for decontamination, rinsing, then wiping with sterile gauze soaked in 70% alcohol. Consult specific manufacturer's recommendations with respect to disinfectant concentration, contact time and method of application.

11. Develop a detailed worksheet outlining all supplies, dose calculations, and procedures and have all necessary supplies on-hand before beginning preparation.

12. Transport the agent from the -20°C or colder freezer to the work area in leak proof bag.

13. Place a puncture-resistant, leak-proof biohazard sharps container in the biosafety cabinet. Place all waste materials (e.g., the original vials containing the virus and all syringes, needles, and needle covers which come in contact with the virus) in this container after dose preparation.

14. Wipe or spray items used for dose preparation with 70% alcohol before placing in the biological safety cabinet. Leave disinfectants in contact with surfaces for at least five minutes before dose preparation. Avoid exposing the virus to disinfectants.

15. Wipe the final dispensing container (e.g., syringe or IV bag) containing the prepared dose with 70% alcohol before removing it from the biological safety cabinet; transport it in a biohazard-labeled leak proof bag or container.

16. Following dose preparation, decontaminate the biological safety cabinet again as outlined in step 10 and dispose of personal protective apparel in a biohazard safety bag or container for disposal.

17. Incinerate all waste materials according to institutional policies and local, state, and federal regulations for infectious biohazardous waste.

18. Handle accidental spills according to institutional biosafety policy, with the following recommendations:

- Prevent others from entering the area and allow aerosols time to settle.
- Use protective apparel: gown, eyewear, mask, and gloves.
- Cover spills with disposable absorbent towels.
- Decontaminate the area with a minimum of 2% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing appropriate contact time.
- Dispose of all waste and protective apparel as infectious biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.
- Immediately report spills and accidents resulting in overt exposure to the IBC. Provide medical evaluation, surveillance, and treatment as appropriate and maintain written records of the event.

Precautions for Healthcare Workers

To date, there have been no reports of viral infection among healthcare professionals preparing or administering the agent. In consultation with the Institutional Biosafety Committee, clinical trial sites should consider the following precautions:

• Avoid contact with the agent if you are pregnant, breast-feeding or immunocompromised (by disease or therapy).
• Adhere to Biosafety Level 2 precautions and wear gloves, gown, mask and eye protection at all times during preparation of Reolysin® and handling contaminated equipment.
• Adhere to appropriate universal, infection control, and biohazard precautions when administering the agent and handling liquid waste (e.g., urine, stool, vomit, sputum) or collecting or handling laboratory samples (e.g., blood, urine). Dispose of all patient liquid waste (e.g., urine, stool, vomit, sputum) in a toilet with ½ cup of bleach, wait 10 minutes prior to flushing, followed by an additional ½ cup of bleach when the water level returns to normal.
• Dispose of all waste generated during administration of the agent in a biohazard bag or container as infectious biohazardous waste. Incinerate according to institutional policy and according to local, state, and federal regulations.
• Decontaminate any contaminated non-disposable equipment and work surfaces with a bleach solution following dose administration.
• Place contaminated non-disposable clothing or linens in a labeled biohazard bag for laundering according to institutional policies for biohazardous items.

Patient Care Implications
Pathogenic effects of reovirus appear to be minimal in immunocompetent individuals, normally producing no or minor illness. Community acquired reovirus infections are restricted to the upper respiratory and gastrointestinal tracts and can be characterized by flulike symptoms, including malaise, rhinorrhea, cough, sneezing, pharyngitis, headache, and loose stool, with an onset of symptoms in 24-48 hours and lasting from 4-7 days. Isolated cases of more serious potentially viral-associated adverse effects exists, including hepatobiliary, neurological, respiratory or exanthematous disease, but a causative association with reovirus infection is unproven.

Viral shedding analysis conducted by Oncolytics Biotech Inc., the manufacturer of REOLYSIN, provides the following information:
Upon administration, the virus rapidly distributes and can be found in tumor, serum, sputum, urine, and feces. Shedding tests using a sensitive RT-PCR assay occasionally showed positive results in urine (and/or less commonly in saliva or feces). It is important to note that the positive test results are only indicative of detection of viral genome rather than active virus. However, since even a negative shedding test does not rule-out minimal viral shedding, standard precautions listed below are recommended. The reovirus utilized in REOLYSIN is a non-manipulated wild-type RNA virus which has never been shown to cause significant illness in humans. In clinical studies to date, using standard precautions, there have been no safety problems regarding the spread of virus from patients to other persons, including other patients, family members or health-care staff. See Appendix B for patient instructions regarding standard precautions. Patients should avoid taking acetaminophen with Reolysin®. Whenever suitable, physicians should utilize alternative medications, such as ibuprofen or aspirin.

Adverse Events and Potential Risks
A list of the adverse events and potential risks associated with Reolysin can be found in Section 7.1.

Availability
Reolysin is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI. Reolysin is provided to the NCI under a Collaborative Agreement between Oncolytics Biotech Inc. and the DCTD, NCI (see Section 12.3).

Agent Ordering
NCI supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Agent may be requested by completing a Clinical Drug Request (NIH-986) and faxing it to the Pharmaceutical Management Branch at (301) 480-4612. For questions about drug orders, transfers, returns, or accountability call (301) 496-5725 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov.

Agent Accountability
The Investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form. *See the CTEP web site for Policy and Guidelines for Accountability and Storage of Investigational Drugs (http://ctep.cancer.gov/requisition/storage.html).*

8.2 Other Investigational Agent(s)

N/A

8.3 Commercial Agent(s)

8.3.1 Carboplatin

Please refer to the FDA approved package insert for complete information on carboplatin.

Product Description:
Biological, Physical and Chemical Characteristics:

Descriptive Name: Carboplatin
Trade Name: PARAPLATIN®

Availability:
Carboplatin is available commercially.

Dose Form, Stability and Packaging:

Carboplatin aqueous solution for injection is supplied as a sterile, pyrogen-free, 10 mg/mL aqueous solution of carboplatin.

Solution Preparation:
Carboplatin will be prepared and administered according to institutional guidelines.

8.3.2 **Paclitaxel**

Please refer to the FDA approved package insert for complete information on paclitaxel.

Product Description:
Biological, Physical and Chemical Characteristics:

Descriptive Name: Paclitaxel
Trade Name: TAXOL®

Availability:
Paclitaxel is available commercially.

Dose Form, Stability and Packaging:
Paclitaxel (TAXOL) for injection is a clear, colorless to slightly yellow viscous solution. It is supplied as a nonaqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. TAXOL is available in 30 mg (5 mL), 100 mg (16.7 mL), and 300 mg (50 mL) multidose vials. Each mL of sterile nonpyrogenic solution contains 6 mg paclitaxel, 527 mg of purified Cremophor® EL* (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol, USP.

Solution Preparation:
Paclitaxel will be prepared and administered according to institutional guidelines.

9 CORRELATIVE/SPECIAL STUDIES

9.1 Laboratory Correlative Studies

9.1.1 Assessment of genetic mutations and/or protein levels of the RAS signaling pathway activation:

9.1.1.1 Collection of Specimen(s)

Patients are required to have tissue available before enrolling on the study. A new biopsy will not be required strictly for purposes of this correlative study. Paraffin-embedded tissue will be obtained from OSU Department of Pathology via the Histology Core Facility as per standard.

Archived paraffin embedded and formalin fixed (FFPE) tissues of previously biopsied or resected specimens are needed for the purposes of:

-

- Pathological examination (for conventional pathology examination, such as H&E stain)
- Molecular pathology for KRAS mutation
- Additional Immunohistochemical stains (for RAF, MEK1/MEK2, ERK1/ERK2) may be performed following KRAS analysis.

Prioritization of correlative studies is unlikely to be necessary since the quantity of tissue required for study will not be a limiting factor.

9.1.1.2 Handling of Specimens(s)

These tissues will be prepared as formalin fixed paraffin embedded (FFPE) blocks and used to produce 1 H&E cut at 4 micrometers, 8 unstained sections, cut at 4 micrometers and mounted in poly-l-lysine-coated or plus (+) slides, and 8 sections containing at least 50% tumor cut at 10 micrometers and mounted on glass slides. If the tissue is small (< $1cm^2$), 20 unstained sections cut at 10 micrometers of thickness will be required. These latter slides will be air dried (not oven-dried). The slides will be stored at room temperature (>16°C). The sectioning of FFPE tissue for the immunohistochemical studies, and molecular study will be done at the same time as the H&E section and the immunohistochemical sections to minimize tissue loss.

Remaining tissue will be archived by the Tissue Archives services at the Pathology Department.

It is anticipated that most patients will have the diagnosis of pancreatic adenocarcinoma either by a prior biopsy or resection. Outside available material will be retrieved from the original institution and reviewed. This material will be reviewed

at OSU by the Department of Pathology. An assessment of the H&E sections will be done to establish the presence of tumor in specific blocks.

Institutions that do not release blocks.
Some institutions have local policies, and according to their policies do not release tissue blocks to other institutions. In these cases, unstained sections mounted in glass slides will be obtained from them. These institutions and laboratories will be furnished with the consent and protocol if this is requested.

Histology confirmation requirements. 1 unstained section cut at 4 micrometers for H&E of each block to be tested.

Immunohistochemical stains requirements. A minimum of 8 slides, cut at 4 microns unstained sections in poly-l-lysine-coated or plus (+) slides, will be required for the RAF, MEK1/2 , and ERK1/2.

KRAS mutation analysis. The minimum requirements are 20 unstained sections cut at 10 micrometers if the tissue is small (< $1cm^2$); and only 8 unstained sections cut at 10 micrometers if the tissue is large.
For these molecular studies, multiple tissue sections can be collected on one glass slide, as the tissue itself is digested and scraped off the slide, thus there is not a need to mount one section per slide. The slides will be air dried (not oven-dried). The slides will be stored at room temperature (>16°C).

This outside available material will be received from the original institution to the address below. The research slides and material will be reviewed at the OSU Pathology laboratories by a Board Certified Pathologist in the Department of Pathology at OSU Medical Center/James Cancer Center.

9.1.1.2.1. Immunohistochemistry

Pre-treatment tumor specimens will be evaluated for relevant pathway activation using immunohistochemistry (IHC). FFPE sections at 4 micrometes mounted in glass slides will be probed with appropriate antibodies and stained to evaluate relative levels of activation. Activation of the Ras pathway is expected to correlate with reovirus infiltration and replication, and therefore we anticipate Ras pathway activation to be a component in predicting response to Reolysin® therapy. Immunoblotting and IHC methods are in common use to evaluate the activation status of the *Ras* pathway [47-51] including evaluations of RAS pathway activation with respect to reovirus infiltration and replication [52-55]. These studies will be performed by the Solid Tumor Translation Science Resource at The Ohio State University.

To evaluated for activated *Ras* pathway, pre-treatment tumor specimens

will be tested RAF, pMEK1/2 and pERK1/2. Other potential markers will be included.
- RAF (BRAF) expression will be performed with a commercially available assay. E.g rabbit monoclonal antibody to Raf (EP152Y, ABCAM)
- The p-MEK1 and p-MEK2 expression level will be studied using another commercially available antibody. E.g, rabbit monoclonal antibody to phospho-Mek (p-Mek1/2; Ser221 antibody ,166F8 , Cell signaling)
- ERK (extracellular signal regulated kinase), also known as the MAPK (mitogen activated protein kinase), expression will be studied with a commercially available assay. E.g. rabbit monoclonal antibody to the active, dually-phosphorylated form of MAP kinase (ERK-1 and ERK-2; Phospho-p44/42 MAPK , Erk1/2, Thr202/Thy204, 20G11; Cell Signaling)

The study will concentrate in the proteins described above. We anticipate encountering a predictor marker in this set of Ras related proteins. However, other markers of interest include PKA, PI3K, Akt, and JAK/STAT. We plan to evaluate these markers to identify a potential predictor of susceptibility of resistance to the combination Reolysin® and chemotherapy.

9.1.1.2.2. Tumor Genetics

Although *Ras* pathway activation is anticipated as a precursor for Reolysin® activity, the source of activation and/or activation of parallel pathways (e.g. PI3-kinase or JAK/STAT) may influence the activity of the Reolysin®/chemotherapy combination. We will focus our initial evaluations on the common pathway activating mutations in *Kras* (codons 12, 13 and 61), which are expected to be prevalent in this study. Genomic DNA will be extracted from 8 to 20, 10-micrometers-thick, sections of formalin fixed paraffin embedded (FFPE) tissue using a commercially available DNA preparation kit (Qiagen DNA preparation kit; Qiagen, Hilden, Germany). The target sequences containing the target mutations will be amplified by Polymerase chain reaction (PCR) with primers that are specific for the gene of interest. The primers will be designed in such a way that they anneal to a sequence which is 100-200 bp 5' of the site where mutations in the *KRAS* gene are expected. After the target DNA synthesis, an additional PCR amplification will be performed with specific primer pairs that amplify a fragment of about 250 bp, which contains the expected mutational sites. The PCR products will be purified to remove excess nucleotides. The purified fragments will be sequenced to determine the mutational status of the protein using ABI 3130*xl* DNA analyzer located at the CLIA certified Molecular Pathology Laboratory (Pathology Core Facility of The OSU Medical

Center). The results of the mutations will be read and interpreted by a pathologist (Dr. W. Zhao, M.D.), and sequencing files will be filed for the purpose of publication or evidence for future use.
Other mutations or gene amplifications (e.g. EGFR amplification) may also be evaluated to better characterize the source of activation for the *Ras* or other pathways.

9.1.1.3 **Shipping of Specimen(s):**

Send the specimen submission form with the each specimen including the patient's initials, institution, date, protocol number and the tissue source.

FFPE specimens should be packaged in a slide holder and shipped in a well cushioned box. These specimens with the relevant pathological report/s can be sent by regular US mail or UPS ground. Slides should be labeled with the protocol number and the patient's initials, date, and institution.

The FFPE specimens should be shipped to:
Cynthia Timmers, Ph.D.
The Ohio State University Comprehensive Cancer Center
BRT Rm 460B
460 West 12th Avenue
Columbus, OH 43210
Ph: 614-366-9041
Cell: 414-617-5466
Cynthia.timmers@osumc.edu

9.1.1.4 **Site(s) Performing Correlative Study**

The Ohio State University will be the central site performing these correlative studies.

9.1.2 **Assessment of the modulatory effect of co-administered chemotherapy with Reolysin® on NARA titers, and of chemotherapy with or without Reolysin® on the inflammatory cytokine profile, and immune effector cell phenotype function and viral persistence**

9.1.2.1 **Collection of Specimen(s)**

Blood samples will be collected prior to treatment on Days 1, 4 and 14 (± 1 day) of Cycle 1; and Day 1 of Cycles 3, and 12 for patients receiving carboplatin plus paclitaxel with Reolysin®. For patients receiving only carboplatin and paclitaxel, blood samples will be collected on Days 1 and 14 (± 1 day) of Cycle 1 and Day 1 of Cycles 3 and 12. Three 10mL green top tubes of blood containing sodium heparin as

an anti-coagulant will be obtained for correlative studies at each of these time points. Blood samples must be shipped at room temperature within 24 hours of the blood draw by courier to the central site (OSU). Blood samples will be centrifuged at room temperature at 805 x *g* for 10 minutes and the plasma layer will be aspirated with a sterile pipet, aliquoted, snap frozen (dry ice or liquid nitrogen) and stored at ≤-70°C until analysis. Plasma samples from individual patient cohorts will be batched and analyzed simultaneously.

9.1.2.2 **Handling of Specimens(s)**

9.1.2.2.1. NARA assay

We hypothesize that carboplatin and paclitaxel will impair the generation of NARA upon reovirus administration. To determine a suitable virus dilution for subsequent NARA assay, L929 cells will be used as targets and Reoviral stock (RT3D) will be used in two fold dilution series such that the final dilutions of the two series were 1:204 800 and 1:10.12 After 2 h, the RT3D inoculums will be removed and replaced with growth medium. After a further 48 h, cell survival will be measured by MTT assay. To establish a suitable dilution series for the estimation of neutralizing antibody levels in the clinical plasma specimens, the above experiment will be repeated with a constant titer of RT3D (known to cause 80% cell death) that is reinsulated with a dilution series of goat polyclonal antireoviral antibody. Experiments will be performed with 2-, 3-, 4- and 10-fold dilution series of the goat polyclonal antibody and cell survival will be measured at 48 h by MTT assay. For experiments with the clinical plasma samples, a positive control of goat polyclonal antibody will always be included and the patient sera will be initially heat-inactivated at 56°C for 30 min.

9.1.2.2.2. Analysis of cytokines in patient plasma.

Although we expect that chemotherapy with carboplatin plus paclitaxel will suppress NARA production via its direct effects on B lymphocytes, prior reports have shown that this regimen can augment the anti-tumor response via a temporary reconstitution of cytotoxic immune effector cells (Prestwich RJ J. Immunol, 2009). Reovirus infection of tumor cells can result in inflammation and serve as an “immune adjuvant” to release specific tumor antigens at the sites of metastasis. The local inflammatory reaction could potentially overcome tumor associated immune suppression and promote epitope spreading from an immune response to the virus to an immune response to the tumor. Indeed, analysis of the innate and adaptive immune responses to reovirus infection has demonstrated that levels of cytotoxic effector NK and T cells can increase upon infection. Alternatively, this pro-inflammatory cytokine profile can aid B lymphocytes in the production of antibodies directed

against the reovirus. Recent pre-clinical studies have demonstrated that single agent cisplatin can down-regulate production of pro-inflammatory cytokines following reovirus infection in vitro and in vivo. Based on these data, cytokine profiles will be monitored in patients enrolled in this trial. Although serial biopsy at the tumor site would be ideal to assess immune reactivity, this is not a clinically feasible option. Therefore, peripheral blood represents a reasonable surrogate for monitoring systemic changes in the inflammatory cytokine profile during the course of treatment. The Th1 and Th2 cytokines including IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IFN-γ and TNF- α cytokines will be detected by the BD Biosciences Cytometric Bead Array (CBA) Inflammation or Th1/Th2 kits and analyzed on a BD FACS caliber flow cytometer. IL-13, IL-17, IFN-α, IFN-β will be detected by commercially available sandwich ELISA.

9.1.2.2.3. Effects of Reolysin and/or Chemotherapy on cancer-associated immunosuppressive cell populations.

Elevated levels of pro-inflammatory cytokines and immunosuppressive cell populations are present in patients with cancer. Our group has previously conducted pilot studies and demonstrated that this profile is also operative in peripheral blood from patients with pancreatic cancer. For example, plasma IL-6, IL-10 and MDSC were elevated in patients as compared to normal donors. These factors represent an important barrier that likely limits the full potential of immune-based therapies or endogenous host responses to tumors. We hypothesize that this state of elevated immune suppressor cells in cancer patients can be manipulated to provide an advantage for the maximal oncolytic effect of reolysin. For these experiments, multiparameter flow cytometry will be utilized to phenotype immune effector cells to determine whether CP downregulate T regulatory cells (CD4+CD25+Foxp3+) and myeloid derived suppressor cells (CD33+HLADR-CD15+CD11b+) as these populations can neutralize host anti-tumor or anti-viral responses and could be modulated in response to chemotherapy. All data will be expressed as the percentage of total circulating peripheral blood cells with each respective phenotype. Appropriate fluorochrome labeled isotype control antibodies will be utilized for determination of background staining and for compensation. The phenotypes chosen represent the most well-accepted and published phenotypic definitions to date, and will be modified as concordance among the tumor immunology community becomes more apparent [57].

9.1.2.2.4. Effects of Reolysin and/or Chemotherapy on lymphocyte profiles and cytotoxic effector cell phenotype.

In addition to its effects of B lymphocytes, reovirus can upregulate the activation and cytotoxic properties of NK cells and CD8+ T cells (Prestwich J Immunol, 2009). Therefore, we will analyze the overall level of B cells (CD19+, CD20+, CD21+) or other immune effectors (CD4+, CD8+, CD56+, CD45RO+ that could serve as a source of immunomodulatory cytokines. In addition, the levels of IFN-γ and cytotoxic effector machinery (granzyme b; perforin) will be measured in cytotoxic NK cells and CD8+ T cells by dual-parameter, intracellular flow cytometry as previously described (Parihar, R., Clin Cancer Res., 2004). These data will allow for a determination as to whether cells from patients receiving Reolysin® are primed to become more cytotoxic as compared to patients treated with carboplatin and paclitaxel alone.

9.1.2.2.5. Effects of Reolysin and/or Chemotherapy on viral persistence

Analysis of viral persistence in plasma and circulating PBMCs will be conducted via PCR using reovirus cDNA-targeted primer sets as previously described (Adair RA et al., Sci Transl Med 2012), Negative (RNase-free H_2O) and positive (reovirus RNA) controls will be included. All samples will be resolved on a 2% agarose gell and analyzed for densitometry via Image J software.

9.1.2.3 Shipping of Specimen(s):

Whole blood should be packaged appropriately and shipped by overnight carrier on Monday, Tuesday, Wednesday or Thursday. Shipments should not be made on Friday. Deliveries on weekends and holidays will not be accepted. Ship blood specimens at room temperature to the following address:

The Ohio State University
Department of Pathology
Human Tissue Resource Network
Attn. Yufang Tang, Ph.D.
2001 Polaris Parkway, Lab #1315
Columbus, OH 43240
(614)-366-6562)

9.1.2.4 Site(s) Performing Correlative Study

The Ohio State University will be the central site performing these correlative studies.

10 STUDY CALENDAR

Baseline clinical and laboratory evaluations are to be conducted within 1 week prior to administration of protocol therapy. Baseline radiographic scans must be done within 3 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating,

laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

10.1 Arm A: Reolysin®, Carboplatin, and Paclitaxel

TEST / DAY	Pre-study (≤ 3 wks)	Cycle 1							Cycles 2, 3, 4, 5 etc.						End of Study
		Pre-Cycle (≤ 1 wk)[14]	Day 1 (± 1 day)	Day 2	Day 3	Day 4	Day 5	Day 14 (± 1 day)	Pre-Cycle (≤ 1 wk)[14]	Day 1 (± 1 day)	Day 2	Day 3	Day 4	Day 5	
Physical exam	X	X							X						X
Complete history	X														
Demographics	X														
Weight	X		X							X					X
Performance status (ECOG)	X		X							X					X
Tumor evaluation[1]	X[1]								X[1]						X[1]
Toxicity assessment			X						X	X					X
Vital signs[2]	X		X	X	X	X	X			X	X	X	X	X	X
Urinalysis[3]		X							X	X					X
CBC and platelets[4]		X							X	X					X
Coagulation	X														
LFTs[5]		X							X	X					X
Serum chemistry[6]		X							X	X					X
Correlative research specimens[7]			X[7]			X[7]		X[7]		X[7]					
Pregnancy test[8]		X								X					X
HIV/Hepatitis test	X														
ECG and troponin levels[10]	X														
Serum CA 19-9[11]	X		X							X					X
Premedication[12]			X							X					
Paclitaxel/carboplatin [13]			X							X					
REOLYSIN Administration			X	X	X	X	X			X	X	X	X	X	

Key:

[1] Tumor evaluation by CT as medically indicated. Evaluation to be performed: pre-study within 3 weeks of treatment, every 8 weeks while on study, and at the end of study visit.

[2] Vital signs (i.e., blood pressure, temperature, heart rate) will be charted starting prior to the paclitaxel infusion.

[3] Specific gravity, pH, protein, glucose, ketones, urobilinogen, blood, microscopic & nitrite.

[4] Total WBC count differential, Hemoglobin, Hematocrit, RBC count, and Platelet count.

[5] AST, ALT, alkaline phosphates, direct and total bilirubin.

[6] Na, K, Cl, HCO_3, BUN, Cr, Glu, Ca, PO_4, T. protein, albumin, Mg, LDH, GGT (pre-study only), uric acid, and troponin I (pre-study only as clinically indicated).

[7] Three 10mL green top heparin tubes of blood will be obtained for correlative studies (NARA levels, B and T cell counts, and cytokine levels as described in section 9). Samples will be obtained prior to treatment on Days 1, 4, and 14 of Cycle 1; and Day 1 of Cycles 3 and 12.

[8] If applicable, must be negative within 7 days of first dose.

[9] [Deleted in amendment 1]

[10] ECG will only be performed prior to study entry and troponin levels only pre-study as clinically indicated.

[11] Serum CA 19-9 levels will be obtained at baseline and prior to each treatment cycle.

[12] Premedication consists of dexamethasone 20 mg p.o. administered approximately 12 and 6 hours before paclitaxel, diphenhydramine (or its equivalent) 50 mg i.v./p.o. 30-60 minutes prior to paclitaxel, and cimetidine (300 mg) or ranitidine (50 mg) i.v./p.o., or equivalent 30 to 60 minutes prior to paclitaxel.

[13] Paclitaxel (following premedication) and carboplatin administration on Day 1 of each cycle. Ideally the three infusions on Day 1 should be scheduled in succession.

[14] The pre-cycle treatment visit may be performed on day 1 of each cycle prior to receiving treatment.

10.2 Arm B: Carboplatin, and Paclitaxel

TEST / DAY	Pre-study (≤ 3 wks)	Cycle 1			Cycles 2, 3, 4, 5 etc.		End of Study
		Pre-Cycle (≤ 1 wk)[14]	Day 1 (± 1 day)	Day 14 (± 1 day)	Pre-Cycle (≤ 1 wk)[14]	Day 1 (± 1 day)	
Physical exam	X	X			X		X
Complete history	X						
Demographics	X						
Weight	X		X			X	X
Performance status (ECOG)	X		X			X	X
Tumor evaluation[1]	X[1]				X[1]		X[1]
Toxicity assessment			X		X	X	X
Vital signs[2]	X		X			X	X
Urinalysis[3]		X			X	X	X
CBC and platelets[4]		X			X	X	X
Coagulation	X						
LFTs[5]		X			X	X	X
Serum chemistry[6]		X			X	X	X
Correlative research specimens[7]			X[7]	X[7]		X[7]	
Pregnancy test[8]		X				X	X
HIV/Hepatitis test	X						
ECG and troponin levels[10]	X						
Serum CA 19-9[11]	X		X			X	X
Premedication[12]			X			X	
Paclitaxel/carboplatin[13]			X			X	

Key:

[1] Tumor evaluation by CT as medically indicated. Evaluation to be performed: pre-study within 3 weeks of treatment, every 8 weeks while on study, and at the end of study visit.

[2] Vital signs (i.e., blood pressure, temperature, heart rate) will be charted starting prior to the paclitaxel infusion.

[3] Specific gravity, pH, protein, glucose, ketones, urobilinogen, blood, microscopic & nitrite.

[4] Total WBC count differential, Hemoglobin, Hematocrit, RBC count, and Platelet count.

[5] AST, ALT, alkaline phosphates, direct and total bilirubin.

[6] Na, K, Cl, HCO_3, BUN, Cr, Glu, Ca, PO_4, T. protein, albumin, Mg, LDH, GGT (pre-study only), uric acid, and troponin I (pre-study only as clinically indicated).

[7] Three 10mL green top heparin tubes of blood will be obtained for correlative studies (B and T cell

counts , and cytokine levels as described in section 9). Samples will be obtained prior to treatment on Days 1 of Cycle 1; Day 14 on Cycle 1; and then prior to treatment on Day 1 of Cycles 3 and 12.

[8] If applicable, must be negative within 7 days of first dose.

[Deleted in amendment 1]

[9] [Deleted in amendment 1]

[10] ECG will only be performed pre-study and troponin levels only pre-study as clinically indicated.

[11] Serum CA 19-9 levels will be obtained at baseline and prior to each treatment cycle.

[12] Premedication consists of dexamethasone 20 mg p.o. administered approximately 12 and 6 hours before paclitaxel, diphenhydramine (or its equivalent) 50 mg i.v./p.o. 30-60 minutes prior to paclitaxel, and cimetidine (300 mg) or ranitidine (50 mg) i.v./p.o., or equivalent 30 to 60 minutes prior to paclitaxel.

[13] Paclitaxel (following premedication) and carboplatin administration on Day 1 of each cycle. Ideally the two infusions on Day 1 should be scheduled in succession.

[14] The pre-cycle treatment visit may be performed on day 1 of each cycle prior to receiving treatment.

11 MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 8 weeks.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, Version 1.1 [58]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with carboplatin, and paclitaxel with or without Reolysin®,

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥10 mm with spiral CT scan (CT scan slice thickness no greater than 5 mm) Malignant lymph nodes will be considered measurable if they are ≥15 mm in short axis.

Note: Tumor lesions that are situated in a previously irradiated area will not be considered measurable.

Non-measurable disease. All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with P10 to <15mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Target lesions. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression'. In addition, it is possible to record multiple nontarget lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

11.1.3 **Methods for Evaluation of Measurable Disease**

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and ≥10mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung. See Appendix II for more details. CT, MRI: CT is the best currently available and

reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less. As is described in Appendix II, when CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). More details concerning the use of both CT and MRI for assessment of objective tumor response evaluation are provided in Appendix II.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next (described in greater detail in Appendix II). If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response.

11.1.4 **Response Criteria**

11.1.4.1 **Evaluation of Target Lesions**

Complete Response (CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR):	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters
Progressive Disease (PD):	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative

increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.4.2 **Evaluation of Non-Target Lesions**

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).

Incomplete Response/ Stable Disease (SD): Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

When the patient also has measurable disease, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare. In such circumstances, the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 **Evaluation of Best Overall Response**

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on

the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration"*. Every effort should be made to document the objective progression even after discontinuation of treatment.

11.1.5 **Duration of Response**

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

11.1.6 **Progression-Free Survival**

PFS is defined as the duration of time from start of treatment to time of documented progression or death whichever occurs first.

11.1.7 **Overall Survival**

Time from study initiation to time of death will be followed and documented for all patients.

12 DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/cdus.htm). **Note**: **All** adverse events that have occurred on the study, including those reported through CTEP-AERS, must be reported via CDUS.

12.1.2 Responsibility for Data Submission

Study participants are responsible for submitting CDUS data and/or data forms to the Coordinating Center quarterly by April 1, July 1, October1, and January 1 to allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP (see **12.1.1.**).

The Coordinating Center is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.2 CTEP Multicenter Guidelines

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the Coordinating Center (Study Coordinator) and the procedures for auditing are presented in Appendix C.

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.

- Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) (except for Group studies).

- AEs will be reported from the participating institutions directly to CTEP as well as to OSU.

12.3 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, Agent-CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http:// ctep.cancer.gov/industry) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements , the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data".):

    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.

    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.

1. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected, used, and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 CFR Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

   Regulatory Affairs Branch, CTEP, DCTD, NCI
   Executive Plaza North, Suite 7111
   Bethesda, Maryland 20892
   FAX 301-402-1584
   Email: ncicteppubs@mail.nih.gov

   The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13 STATISTICAL CONSIDERATIONS

13.1 Study Overview:

This is a randomized phase II study with equal allocation to each of two regimens: Reolysin®, carboplatin, and paclitaxel relative to carboplatin and paclitaxel without Reolysin ® in patients with recurrent or metastatic pancreatic cancer. The primary goal of this study is to assess the improvement in progression-free survival (PFS) in patients treated with Reolysin®, carboplatin, and paclitaxel relative to carboplatin and paclitaxel alone. In a secondary manner we will also evaluate the immunologic effects of adding Reolysin to carboplatin and placlitaxel by evaluating immunologic markers between the two treatment arms.

13.1.1 Primary Endpoint

The primary endpoint to be evaluated in each of the treatment arms is progression-free survival (PFS). PFS is defined as the time from study entry to the date of documented progression and/or death. All eligible patients who have been randomized and begun treatment will be included for the evaluation of the primary endpoint. Patients who discontinue treatment and go on to alternate therapy prior to disease progression will be censored at that timepoint. Alive and progression-free patients will be censored at their last follow-up date.

13.2 Sample Size, Accrual, and Study Duration:

A maximum of 70 eligible and evaluable patients will be enrolled on this trial and randomized in a 1:1 allocation to Reolysin®, carboplatin, and paciltaxel versus carboplatin and paclitaxel alone. Randomization assignment will be centralized, and the study statistician and the clinical trials office will be responsible for generating and assigning the treatment allocations. This randomization scheme will utilize a random permuted blocks approach with varying block sizes. We anticipate that the annual accrual rate will be at least 40 patients; therefore, the accrual period is expected to be approximately 21 months. These estimates are based on previous clinical trial experience in this patient population and practice history as well as the fact that patients will be accrued at the other participating institutions. All patients will be followed for a minimum of 12 months. Final efficacy analyses will begin after all patients have been followed for at least 12 months or until they have a defined event of interest (disease progression and/or death).

13.3 Primary endpoint analysis plans and power:

Prior data in this patient population show that the standard expected median progression-free survival is about 3 months. With the standard chemotherapeutic regimen of carboplatin and paclitaxel, we therefore expect that the median PFS will be approximately 3 months. This study is designed to detect an improvement in the median PFS from 3 months to 5.5 months with the addition of Reolysin® to this

chemotherapeutic regimen. Since we are specifically interested in the improvement in PFS that the addition of Reolysin® adds to the chemotherapeutic regimen, we will use a one-sided test to compare the PFS between the two treatment arms. While we recognize that direct comparisons are not standard in the phase II setting, this analytic approach is gaining greater acceptance through various randomized phase II study designs that allow inclusion of a control arm and allow proof of concept analyses to evaluate not only the clinical outcome benefits but also the impact on correlative endpoints with the addition of a novel agent to a standard regimen. In the randomized phase II setting, especially with a focus on proof of concept, constraining the Type I error up to 0.20 has been proposed in the literature [1-4]. In addition, we feel the likelihood of a Type I error (i.e. concluding that Reolysin added to carboplatin+paclitaxel improves PFS when in fact it does not) is lessened by the addition of the immunological marker analyses that are targeted to determining if the Reolysin is having the effect we expected with differences in immune functions between the two treatment arms.

In this trial, the proposed sample size of 70 eligible and evaluable patients (35 per arm) provides 90% power to detect an improvement from 3 months to 5.5 months in the PFS median with the addition of Reolysin® to carboplatin and paclitaxel over carboplatin and paclitaxel alone. This assumes a one-sided Type I error rate of 0.20, that the accrual period will be about 20 months and the accrual pattern across time periods is relatively uniform, that we will have at least 12 months of follow-up on patients in absence of an event, and that the proportion of patients who drop out of each of the treatment groups is at most 0.05. This sample size is based on a log-rank test calculation using the PASS software [59].

The progression-free survival distributions between the two arms will be compared using log-rank tests, where progression-free survival is as defined above in **13.1.1**. Progression-free survival curves will be constructed using the Kaplan-Meier product limit method, and additional analyses will be done using the Cox proportional hazards model. If a patient on the carboplatin+paclitaxel arm has disease progression, they will be given the opportunity to receive Reolysin® after that point in an effort to open access to the experimental agent to all patients who consent to randomization. However, those patients will be included in the carboplatin+paclitaxel alone arm since they would already have had an event (disease progression), which would not confound the primary endpoint analysis.

13.4 Secondary endpoints and analyses:

13.4.1 Overall response rate will be evaluated using the standard RECIST criteria, where we will evaluate the proportion of patients who have a partial or complete response by treatment group. Assuming the number of patients who have an objective response to their assigned treatment regimen is binomially distributed, 95% confidence intervals will be calculated. In addition, we will assess differences in objective response rates between the treatment arms using Fisher's exact test since the number of patients with a response to treatment is expected to be relatively low.

13.4.2 Overall survival will be defined as the time from study entry to the time of death due to any cause. Patients who are still alive will be censored at their last follow-up date. Overall survival (OS) will be evaluated and compared between the two treatment groups using log-rank statistics and graphically using the methods of Kaplan and Meier. In addition, we will analyze overall survival in two different ways, where we analyze all data assessed for each of the treatment arms (1) using death due to any cause as an event and censor any living patients, and (2) using death due to any cause as an event but also censor those patients who go on to alternate therapies after completing their assigned treatment. This includes those patients on the carboplatin+paclitaxel arm who have disease progression and go on to receive Reolysin®, where those patients will be censored on the date they go on to receive the alternate therapy.

13.4.3 To specifically evaluate the impact of adding Reolysin® to carboplatin and paclitaxel, we will assess and compare the inflammatory cytokine profile, immune effector cell phenotype and function, and in particular NARA titers. Exploratory data analyses will include graphical evaluation of these immunologic correlative markers at baseline as well as at the various timepoints. In addition, we will descriptively summarize the continuous markers quantitatively. Patterns of change in the longitudinal data on these markers will be evaluated in this manner for each of the correlative outcomes of interest. Of particular interest are the NARA titer levels, which will be measured on days 1, 4, and 14 of cycle 1 and day 1 of cycles 3 and 12 for those patients receiving the Reolysin, carboplatin, and paclitaxel treatment combination. We will assess changes in the serum NARA or equivalent titers over time and explore graphically how changes in this marker differ between those with versus without an objective response to therapy as well as other potential factors such as age. The lymphocyte profiles and cytotoxic effector cell phenotypes will be evaluated graphically and descriptively summarized between the two treatment groups, with focus on B cell levels and other immune effectors but also on IFN-γ and granzyme b and perforin as a measure of NK cell activation. In looking at differences in these markers at specific timepoints (e.g. day 14 cycle 1), a two-sided, two-sample t-test will have at least 80% power to detect a moderately large effect size (0.70 s.d.) with 35 patients per treatment group (assuming a significance level of 0.05). Linear mixed models will also be used to explore differences in these markers between the two treatment groups along with graphical analyses that plot marker values across time for the two treatment groups to assess potentially different patterns of change between these groups. Appropriate transformations of the various correlative markers will be used in the presence of skewed data distributions. Multiple comparison corrections will not be used for these secondary correlative analyses.

13.4.4 Descriptive statistics and graphical analyses will also be used to assess data collected from assays evaluating the activation of the Ras pathway, where we will assess the proportion of patients with Ras pathway activation for each treatment group along with corresponding 95% confidence intervals. Whether or not patients had pathway activation will also be summarized by treatment arm and by clinical outcomes. Pathway activation by treatment arms will be explored in relation to PFS using Kaplan-Meier curves. For the clinical outcome of objective response, Cochran-Mantel-Haenszel test will be used to assess differences in the relationships between response and Ras pathway activation and the association of treatment groups on these relationships.

13.5 Toxicity and Tolerability

As per NCI CTCAE v4.0, the term toxicity is defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment. The maximum grade for each type of toxicity will be recorded for each patient, and frequency tables will be reviewed to determine toxicity patterns. In addition, we will review all adverse event data that is graded as 3, 4, or 5 and classified as either “unrelated” or “unlikely to be related” to study treatment in the event of an actual relationship developing. The incidence of severe (grade 3+) adverse events or toxicities will be described for each treatment arm, but will also be compared between

the arms. Fisher's exact tests will be used to quantitatively compare the incidence of severe as well as specific toxicities of interest between the treatment arms and we will graphically assess differences in maximum grades observed for toxicities between the arms. Given the relatively limited number of subjects per treatment group, these comparisons will be exploratory, but useful in identifying potentially toxicity patterns of interest and in planning future trials. We will also assess tolerability of the regimens through assessing the number of patients who required dose modifications and/or dose delays. In addition, we will also capture the proportion of patients who go off treatment due to adverse reactions or even those who refuse further treatment for lesser toxicities that inhibit their willingness to continue participation on the trial. These tolerability measures will be assessed within each of the treatment arms and we will explore differences in these measures between the arms. All patients who have received at least one dose of any of the therapeutic agents in a treatment arm will be evaluable for toxicity and tolerability.

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Informed Consent

The informed consent is attached as a separate document.

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B PATIENT POST-TREATMENT INFORMATION SHEET

The treatment you are receiving is called REOLYSIN. This new treatment uses a naturally-occurring reovirus to treat your cancer. Reovirus is commonly found in wet places such as rivers, ponds and ditches---or in puddles in your yard. In fact, reovirus is occasionally found even in tap water from public water supplies.

Almost all adults have been exposed to, and infected by, reovirus at some time in their lives. This is known because after people are infected they carry antibodies against reovirus. In one study, 75% of children were shown to have been infected with reovirus by the age of 10 years. Most people exposed to reovirus have no symptoms, but a few will have minor symptoms similar to a mild head cold or stomach upset.

Studies have shown that patients treated with reovirus (REOLYSIN) do not show evidence of being significantly "contagious" because the amount of reovirus in their saliva, urine, or stool---even when it can be found at all---is very small.

However, although reovirus usually causes no significant illness, your doctor wants you to take some precautions to minimize the exposure of others:

- You should avoid close contact with severely immune-compromised individuals such as patients who have had a recent bone-marrow or organ transplant or patients with AIDS.

- **On the days that you receive REOLYSIN and for 2 days afterwards,** it is recommended that you adopt the same practices that you would usually observe with family and friends when you have a cold or 'flu'. These recommendations include such things as:

    - Use a separate bathroom at home, if possible, and wash your hands with soap after using the toilet. Clean your toilet more frequently than normal.
    - Avoid sharing cups, drinking glasses or eating utensils.
    - Use detergent to wash the dishes, either by hand or in a dishwasher
    - Avoid close contact (touching) with pregnant women and infants.

You should also avoid taking acetaminophen (Tylenol) or acetaminophen-containing products with REOLYSIN because there is a risk of injury to your liver. Talk to your study doctor about other medicines you can take for fever or aches and pains.

If you have any questions about any of these recommendations, you should ask your study doctors or nurses.

APPENDIX C CTEP MULTICENTER GUIDELINES

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data or study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records,

all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

Inclusion of Multicenter Guidelines in the Protocol

- The protocol must include the following minimum information:
  - The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
  - The Coordinating Center must be designated on the title page.
  - Central registration of patients is required. The procedures for registration must be stated in the protocol.
  - Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms
  - The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
  - Describe how Safety Reports and Action Letters from CTEP will be distributed to participating institutions.

Agent Ordering

- Except in very unusual circumstances, each participating institution will order DCTD supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

APPENDIX D ELIGIBILITY CHECKLIST

NCI 8601: A 3-arm Randomized Phase II Study of Carboplatin, Paclitaxel plus Reovirus Serotype-3 Dearing Strain (Reolysin®) vs. Carboplatin and Paclitaxel in the First Line Treatment of Patients with Recurrent or Metastatic Pancreatic Cancer

Protocol Version: _____Patients Initials: _____

Consent Form Version: _____

Inclusion Criteria All responses must be checked (✓) YES to be eligible.	Yes	No
Patient has histologically confirmed adenocarcinoma of the pancreas that is recurrent or metastatic. Cytological confirmation is not allowed on this study, as tissue is needed for correlative science analysis. Paraffin embedded tissue from tumor blocks will be required from the patient before enrolling on this study. Diagnosis of pancreas cancer with histologic confirmation of adenocarcinoma would suffice.		
Patient has measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension per RECIST 1.1 (longest diameter to be recorded) as ≥10mm with spiral CT scan (CT scan slice thickness no greater than 5mm. Malignant lymph nodes will be considered measurable if they are ≥15mm in short axis. See section 11 for the evaluation of measurable disease. For patients previously irradiated, the measurable lesion must be outside the radiated field.		
Patient has not received any prior chemotherapy in metastatic setting. Patients who have received prior chemotherapy in the adjuvant setting will not be eligible for our study. Patients should not have received prior Reolysin. Prior palliative radiation therapy or major surgery must have occurred at least 28 days prior to study enrollment. Prior minor surgeries (such as laparoscopies) must have occurred at least 14 days prior to study enrollment. Prior minor procedures such as biopsies and mediport placement must have occurred at least 48 hours prior to study enrollment.		
Age ≥ 18 years		
ECOG performance status ≤ 1 (Karnofsky ≥ 70%; see Appendix A).		
Patient has normal organ and marrow function as defined below:		
Absolute neutrophil count ≥ 1.5 x 10^9/L SI units		
Platelets ≥ 100 x 10^9/L SI units		
Hemoglobin ≥ 8.5 g/dl (gm/L) SI units		
Serum Creatinine ≤ 1.5 **or** Creatinine Clearance ≥ 60 mL/min (calculated using Cockcroft-Gault equation)		
Bilirubin ≤ institutional upper limit of normal (≤ 2x ULN if it is non-rising for a period of 10 days prior to initiation of therapy)		
AST/ALT ≤ 3 x institutional upper limit of normal		
Troponin I < institutional upper limit of normal		
Patient has signed an informed consent indicating that they are aware of the neoplastic nature of their disease and have been informed of the procedures of the protocol, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts.		
Patient agrees to use adequate contraception, if applicable. The effects of Reolysin® on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because the other chemotherapeutic agents used in this trial are known to be teratogenic, **women of child-bearing potential and men must agree to use adequate contraception** (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.		
Patient will be able to avoid direct contact with pregnant or nursing women, infants and immunocompromised individuals while on study and for ≥3 weeks following the last dose of Reolysin administration.		
Patient is willing and able to comply with scheduled visits, the treatment plan, and laboratory tests.		

Date Checked _____ Research Staff/PI Initials: _____ / _____

Exclusion Criteria All responses must be checked (✓) NO to be eligible.	Yes	No
Patient is receiving any other investigational agents or concurrent therapy with other anti-cancer agents while on study.		
Patient has untreated brain metastases. (Patients with resected oligometastasis are eligible if postresection MRI demonstrates resolution. Gamma-knife treated patients are also eligible if there are no more than two treated metastases confined to the same area of the brain and a post treatment MRI shows a decrease in the metastases.)		
Patient has history of allergic reactions attributed to compounds of similar chemical or biologic composition to Reolysin® or other agents used in the study.		
Patient has received any viral-based therapy within the past 6 months.		
Patient has continuing acute toxic effects (except alopecia) of any prior radiotherapy, chemotherapy, or surgical procedures. All such effects must have resolved to Common Terminology Criteria for Adverse Events (CTCAE, v.4) Grade ≤ 1 prior to study enrollment.		
Patient has grade 2 or higher baseline peripheral neuropathy, according to CTCAE v.4.		
Patient has uncontrolled cardiac dysfunction or arrhythmia, including a myocardial infarction in the preceding 6 months, known cardiac ejection fraction < 40%, symptomatic congestive heart failure, or unstable angina pectoris.		
Patient is receiving current systemic immunosuppressive therapy		
Patient has known HIV infection or active hepatitis B or C.		
Patient has uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, or known psychiatric illness/ social situations that would limit compliance with study requirements.		
Patient has dementia or altered mental status that would prohibit informed consent.		
Patient has any other known severe, acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or study drug administration or may interfere with the interpretation of study results and, in the judgment of the Principal Investigator, would make the patient inappropriate for this study.		
The patient is a pregnant or lactating woman. Pregnant women are excluded from this study because of the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Reolysin®, breastfeeding should be discontinued while the mother is being treated with the agents in this clinical trial.		

Research Staff: _____
Printed Name

_____ Date: _____
Signature

Principal Investigator: _____
Printed Name

_____ Date: _____
Signature

APPENDIX E REGISTRATION FORM

NCI 8601: A 3-arm Randomized Phase II Study of Carboplatin, Paclitaxel plus Reovirus Serotype-3 Dearing Strain (Reolysin®) vs. Carboplatin and Paclitaxel in the First Line Treatment of Patients with Recurrent or Metastatic Pancreatic Cancer

To: *Jennifer Sexton* **Tel: 614-366-5642**
Fax: 614-366-4721

From: _____ **Fax: _____**
Tel: _____

Subsite Institution: _____
Subsite Principal Investigator: _____
Number of pages _____

Demographic Information:

Patient's Last Name _____

Patient's First Name _____ Middle Initial _____

Date of Birth _____ ☐ Male ☐ Female

Local Medical Record Number _____

Diagnosis _____ ICD-9 code: _____

Ethnicity: ☐ Hispanic or Latino ☐ Not Hispanic or Latino ☐ Unknown

Race:

☐ American Indian or Alaska Native ☐ Asian
☐ Native Hawaiian or Other Pacific Islander ☐ Black/African American
☐ White ☐ More than one race ☐ Unknown or not reported

Attach:

- Eligibility Checklist
- Signed Informed Consent and HIPAA Authorization Form
- Laboratory Tests
- Any relevant source documents required to confirm eligibility

For OSU Use only

Study Subject ID: _____ Registration Date: _____

Eligibility Verifier: _____

Tel: _____

_____ _____
Signature *Date*

APPENDIX F SAE/DATA SUBMISSION FORM

NCI 8601: A 3-arm Randomized Phase II Study of Carboplatin, Paclitaxel plus Reovirus Serotype-3 Dearing Strain (Reolysin®) vs. Carboplatin and Paclitaxel in the First Line Treatment of Patients with Recurrent or Metastatic Pancreatic Cancer

To: ***Jennifer Sexton*** **Tel: 614-366-5642**
Fax: 614-366-4721

From: _____ **Fax: _____**
Tel: _____
Subsite Institution: _____
Subsite Principal Investigator: _____
Number of pages _____

Patient's ID _____ Patient's Initials (Optional):_____

Submission Type

☐ SAE ☐ Data Submission (Cycle _____)

Additional Comments:

Attach:

- Any relevant source documents required to confirm SAE/Data

Supplemental File S1. Methods for Correlative Studies.

Analysis of KRAS mutational status.

Formalin fixed paraffin embedded (FFPE) archival tissue samples were collected at time of study entry. To enrich for tumor content, tumor-containing sections of tissue were macrodissected from regions corresponding to marked H&E stained slides. DNA was purified using the Maxwell 16 System and FFPE Tissue LEV DNA kit (Promega Corp., Madison WI), in accordance with the manufacturer's protocol. *KRAS* codons 12 and 13 mutation detection was carried out using well-established methods of direct DNA sequencing(1) and analyzed using the SeqMan Pro program (DNASTAR, Madison, WI).

Blood procurement

For laboratory correlative studies, three green-top tubes containing sodium heparin as the anti-coagulant were drawn from each patient at baseline and following three cycles of treatment. Peripheral blood mononuclear cells (PBMCs) and plasma were isolated from whole blood via density gradient centrifugation using Ficoll-Paque (Amersham Pharmacia Biotech; Uppsala, Sweden) as previously described(2). PBMCs were cryopreserved in liquid nitrogen and plasma was stored at -80°C until batched analysis.

Cytokine, chemokine, and growth factor analysis

A panel of 32 cytokines, chemokines, and growth factors was analyzed in plasma isolated from patient peripheral blood using Luminex Multiplex Cytokine Kits (Procarta Cytokine Assay, eBioscience; San Diego, CA) as previously described(3). The analytes examined were as

follows: Patient plasma samples were analyzed in duplicate, run in batches, and quantified using analyte specific standard curves for each batch that were provided by the manufacturer.

Flow cytometry

Phenotypic analysis of circulating immune cells was conducted by fluorescence activated cell sorting (FACS). Briefly, cryopreserved PBMCs were thawed rapidly at 37°C, washed, centrifuged, and resuspended in FACS buffer (PBS + 5% FBS). Cells were stained with appropriate fluorochrome-conjugated antibodies in the dark for 45 minutes at 4°C. The following antibodies were utilized for these studies: CD3-FITC (UCHT1, BD Biosciences), CD4-APC (13B8.2, Beckman Coulter), CD4-FITC (OKT4, BioLegend), CD4-Pacific Blue (OKT4, BioLegend), CD8-APC (B9.11, Beckman Coulter), CD11b-PE (Bear1, Beckman Coulter), CD15-FITC (80H5, Beckman Coulter), CD19-APC (HIB19, BioLegend), CD20-BrilliantViolet650 (2H7, BioLegend), CD25-PE (BC96, BioLegend), CD27-AlexaFluor700 (M-T271, BioLegend), CD33-APC (D3HL60.251, Beckman Coulter), CD45RA-PE (HI100, BD Biosciences), CD45RO-PECF594 (UCHL1, BD Biosciences), CD56-AlexaFluor647 (B159, BD Biosciences), CD69-PE (FN50, BD Biosciences), CD71-PE (M-A712, BD Biosciences), CD95-PE (DX2, BD Biosciences), CD127-PE/Cy7 (A019D5, BioLegend), CD134-PE (Ber-ACT35, BioLegend), CD137-PE (4B4-1, BioLegend), CD152-PE (BNI3, BD Biosciences), CD178-PE (NOK-1, BioLegend), CD223-PE (3DS223H, eBioscience), CD272-PE (MIH26, BioLegend), CD279-PE (MIH4, BD Biosciences), CD357/GITR-APC (621, BioLegend), HLA-DR-PE/Cy7 (Immu-357, Beckman Coulter), TIM3-PE/Cy7 (F38-2E2, BioLegend), PE-isotype control #1 (MOPC-21, BioLegend) and PE-isotype control #2 (MOPC-21, BD Biosciences). After incubation, the cells were then washed, fixed in phosphate buffered saline (PBS) + 1% formalin,

and analyzed using an LSRII flow cytometer or FACS calibur (BD Biosciences; San Jose, CA). Compensation controls were generated using ABC capture beads beads (Life Technologies; Eugene, OR). Data were analyzed using FlowJo software version 7.6.4 (FlowJo; Ashland, OR).

Measurement of neutralizing anti-reovirus antibody (NARA) titers.

Neutralizing anti-reovirus antibody (NARA) titer levels were measured on days 1, 4, and 14 of cycle 1, and day 1 of cycles 3 and 12 for those patients receiving pelareorep, carboplatin, and paclitaxel. Antibody titers were detected in samples via a modified NARA assay as described(4). Briefly, L929 cells were used as targets for infection with a constant titer of Reoviral stock (RT3D) that results in 80% cell death with various dilutions of heat inactivated patient plasma. After 48 h, cell survival was measured by MTT assay. Cells incubated with goat polyclonal antireoviral antibody served as a positive control.

QTWiST Method.

After filtering through toxicity grade, type and occurrence period, 66 patients had toxicity data to include in the analysis. Seven patients did not have toxicity data as it was filtered out by using the criteria all grade 3 and 4 toxicities and the following grade 2 toxicities: nausea, fatigue, peripheral neuropathy, diarrhea. To apply the Q-twist method, a patient's overall survival period was partitioned into three parts: toxicity period (TOX), time without symptoms of disease progression or toxicities (TWiST) and relapse (REL). And QTWiST was calculated as the weighted sum of these three durations using the following formula:

Q-twist=Utox*TOX + Urel*REL + Utwist*TWiST

To account for toxicity, all of the grade ≥3 adverse events regardless of attribution were included as well as grade 2 nausea, vomiting, fatigue, diarrhea and peripheral neuropathy. The days spent with these AEs were summed up for each patient, and if an AE was still ongoing at disease progression, the toxicity duration would be capped at the progression date. Any adverse events occurred after disease progression were excluded from the analysis since the method assumes that the three health states are progressive and mutually exclusive.

Time with toxicity, PFS and OS were estimated using Kaplan-Meier method and restricted means were used to calculate the cumulative area under the curves, which represent the duration of each health state in this analysis. Therefore the area under the TOX curve represents mean TOX time, the area between PFS and TOX curves represents mean TWiST time, and the area between OS and PFS curves was the estimation of mean REL time. Nonparametric bootstrap method was used to construct the 95% confidence intervals for these estimates.

The utility coefficient for TWiST was assumed to be 1 and the QTWiST scores from a few different combinations of utility coefficients for TOX and REL were presented.

References.

1. Kim ST, Lim do H, Jang KT, et al. Impact of KRAS mutations on clinical outcomes in pancreatic cancer patients treated with first-line gemcitabine-based chemotherapy. Mol Cancer Ther 2011;10:1993-9.
2. Lesinski GB, Kondadasula SV, Crespin T, et al. Multiparametric flow cytometric analysis of inter-patient variation in STAT1 phosphorylation following interferon Alfa immunotherapy. J Natl Cancer Inst 2004;96:1331-42.
3. Mace TA, Ameen Z, Collins A, et al. Pancreatic cancer-associated stellate cells promote differentiation of myeloid-derived suppressor cells in a STAT3-dependent manner. Cancer Res 2013;73:3007-18.
4. White CL, Twigger KR, Vidal L, et al. Characterization of the adaptive and innate immune response to intravenous oncolytic reovirus (Dearing type 3) during a phase I clinical trial. Gene Therapy 2008;15:911-920.

Supplemental Figures

Supplemental Tables

Supplemental Figure Legends

Figure S1. Schema. For dosing of carboplatin, creatinine clearance was calculated using the Cockroft-Gault equation and total dose was calculated using the Calvert formula. The estimated GFR was capped at 125 ml/min for all patients. Patients were advised to avoid acetaminophen-containing preparations due to possible interaction between pelareorep and acetaminophen and increased risk of hepatotoxicity resulting in increases in liver enzymes (ALT/SGPT and GGT). Granulocyte-colony stimulating factor and/or transfusions of packed red blood cells and platelets were given as clinically indicated, per American Society of Clinical Oncology, National Comprehensive Cancer Network or institutional guidelines.

Figure S2. Overall Survival.

Figure S3 A and B. QTWiST analysis for Arm A (A) and Arm B (B).

Figure S4. QTWiST analysis for both arms combined.

Supplemental Table Legends

Table S1. Univarate and multivariate analysis of PFS and OS by age

(1) HR of treatment arm from univariable Cox model for PFS
(2) HR of treatment arm adjusting for age from two-variable Cox model for PFS
(3) HR of treatment arm from univariable Cox model for PFS
(4) HR of treatment arm adjusting for age from two-variable Cox model for PFS

Table S2. Second-line regimens received following progression.

Table S3. Relationship between plasma immune biomarkers as determined by bioplex analysis with disease control rate (DCR) or progression free survival (PFS). Data indicate the mean fold change in values for all subjects, regardless of treatment arm when comparing values at baseline to those following 3 cycles of treatment.

Table S4. Relationship between cellular immune biomarkers as determined by flow cytometry with disease control rate (DCR) or progression free survival (PFS). Data indicate the mean fold change in values for all subjects, regardless of treatment arm when comparing values at baseline to those following 3 cycles of treatment.

Table S5. Mean Duration of health states by treatment arm. Table S3 shows the restricted mean of the duration of each health state, which is the area under the survival curves. The last column shows the difference between arm A and B, since zero is contained in all of the 95% CI's, there's no significant difference between arm A and B for all of these health state durations.

(1) TOX state comprised the total number of days after randomization and before progression spent with toxicity, regardless of when the toxicity started or whether there were gaps between toxicities. This is measured as the area under the TOX (red) curve. For arm A, the mean TOX duration is 2.42 months with 95% CI of 1.48 to 3.55 months; for arm B, the mean TOX duration is 2.18 months with 95% CI of 1.58-2.8 months. The difference in mean TOX time between arm A and B is 0.24, with 95% CI of -0.88 to 1.56. Since 0 is contained in this 95% CI for difference, we conclude that there's no significant difference in TOX duration between arm A and B.

(2) TWiST is defined as PFS time minus time with toxicities and is represented by the area between PFS (blue) and TOX (red) curves = (4)-(1)

(3) REL state is the duration of relapse and is defined as overall survival time minus PFS time, or the period of time from progression to death. Patients alive at the end of the study were censored for the OS endpoint. The REL state is measured as the area between OS (black) and PFS (blue) curves = (5)-(4)

(4) PFS: the area under the PFS curve

(5) OS: the area under the OS curve

Table S6. Sensitivity analysis for QTWiST. Q-Twist is the weighted sum of the health state durations:

Q-twist=Utox*TOX + Urel*REL + Utwist*TWiST

Table S4 assumes the utility for TWiST equals one (as convention) and lists 9 different scenarios for different values of utility scores for TOX and REL. All of the 9 difference CI's contain zero, so there's no significant difference in Q-twist between arm A and B. The bolded italicized case where the utility score equals 0.5 for both TOX and REL could be viewed as a base case to provide a mid-point for interpretation of QTWiST scores.

Table S7. Mean duration of health states when all patients in the trial were included in the analysis

Table S8. Sensitivity Analysis for all patients

Figure S1

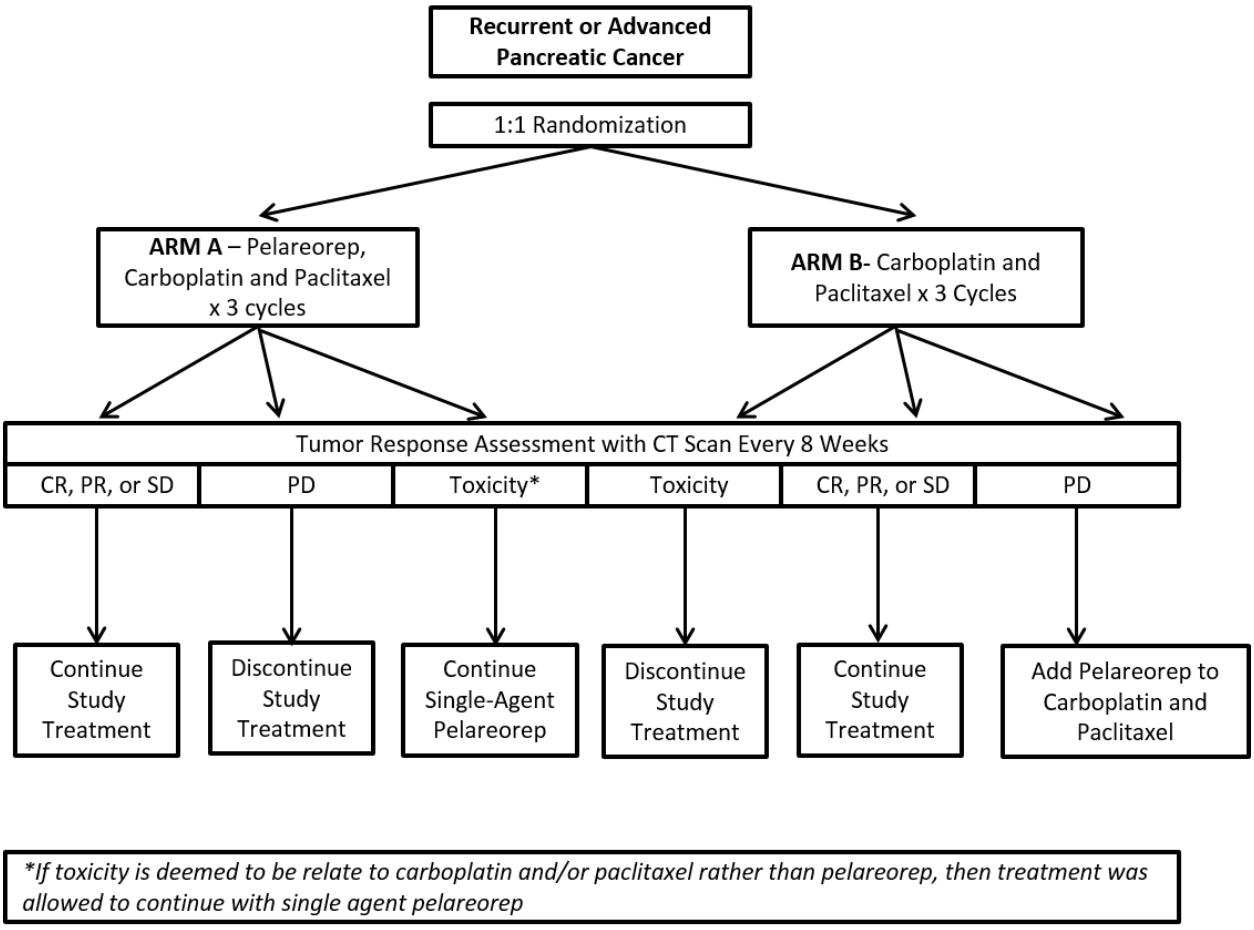

Recurrent or Advanced Pancreatic Cancer
1:1 Randomization
ARM A – Pelareorep, Carboplatin and Paclitaxel x 3 cycles
ARM B- Carboplatin and Paclitaxel x 3 Cycles
Tumor Response Assessment with CT Scan Every 8 Weeks
CR, PR, or SD
PD
Toxicity*
Toxicity
CR, PR, or SD
PD
Continue Study Treatment
Discontinue Study Treatment
Continue Single-Agent Pelareorep
Discontinue Study Treatment
Continue Study Treatment
Add Pelareorep to Carboplatin and Paclitaxel
*If toxicity is deemed to be relate to carboplatin and/or paclitaxel rather than pelareorep, then treatment was allowed to continue with single agent pelareorep

Figure S2

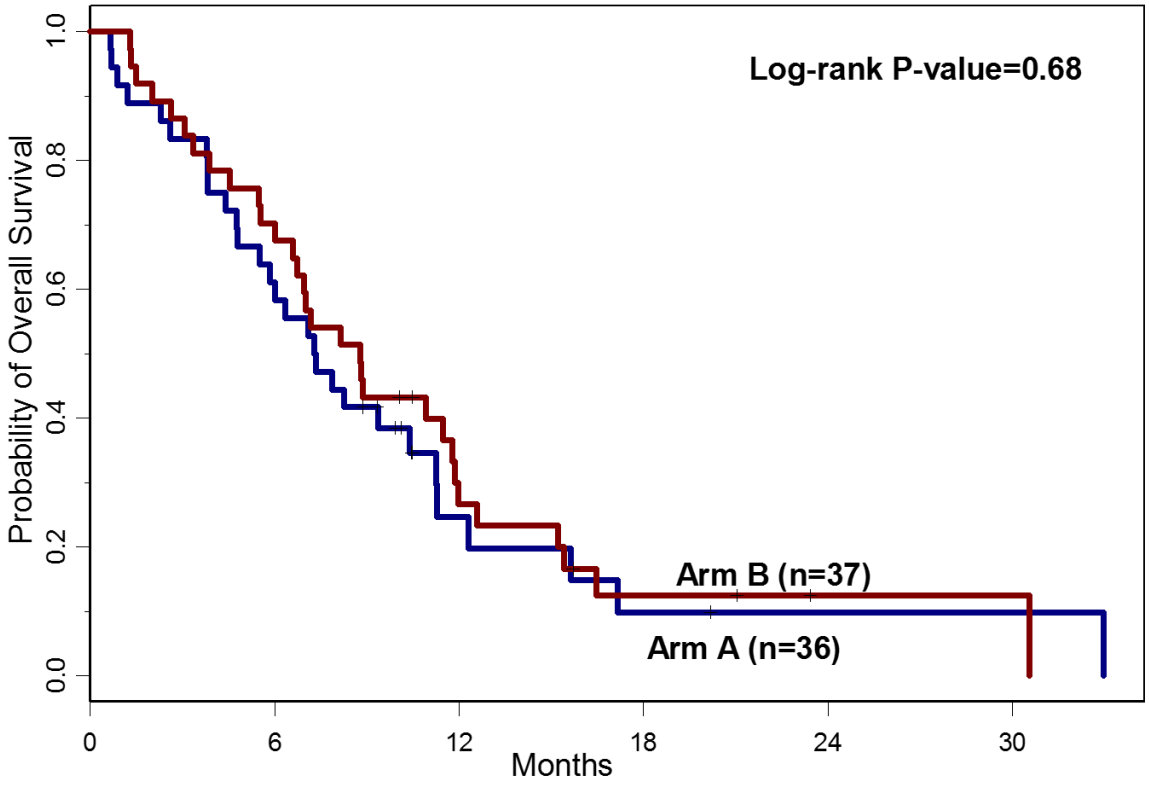
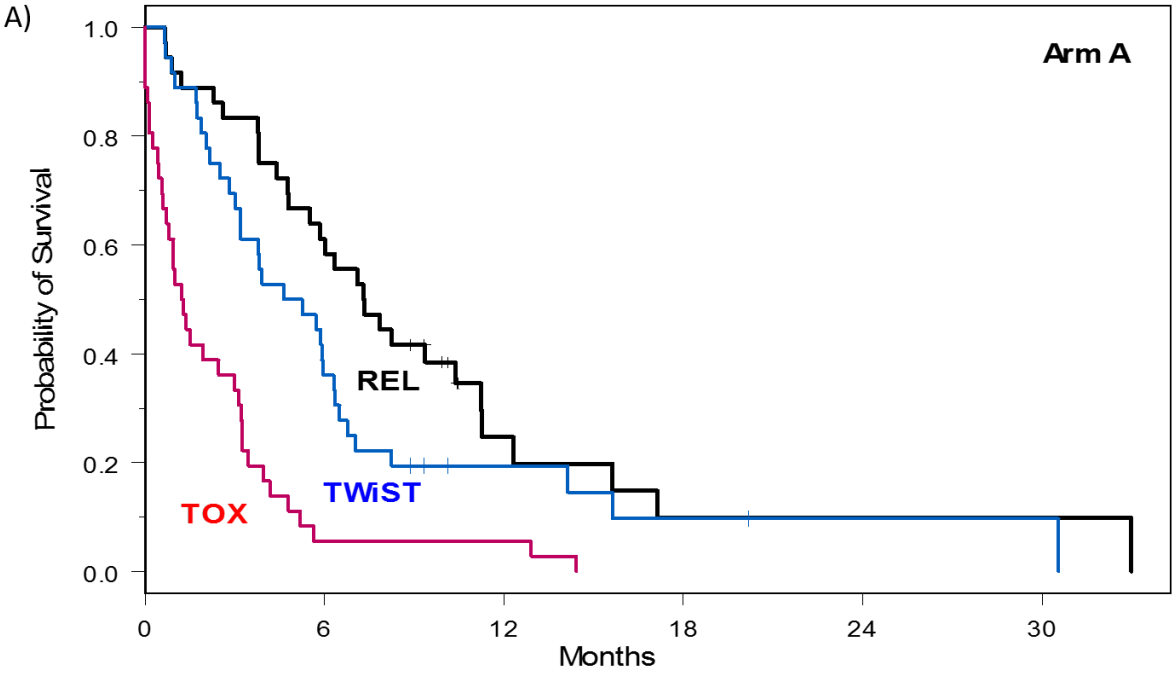

Log-rank P-value=0.68
Arm B (n=37)
Arm A (n=36)
Probability of Overall Survival
0.0
0.2
0.4
0.6
0.8
1.0
0
6
12
18
24
30
Months

Figure S3


A)
Arm A
Probability of Survival
1.0
0.8
0.6
0.4
0.2
0.0
REL
TWiST
TOX
0
6
12
18
24
30
Months

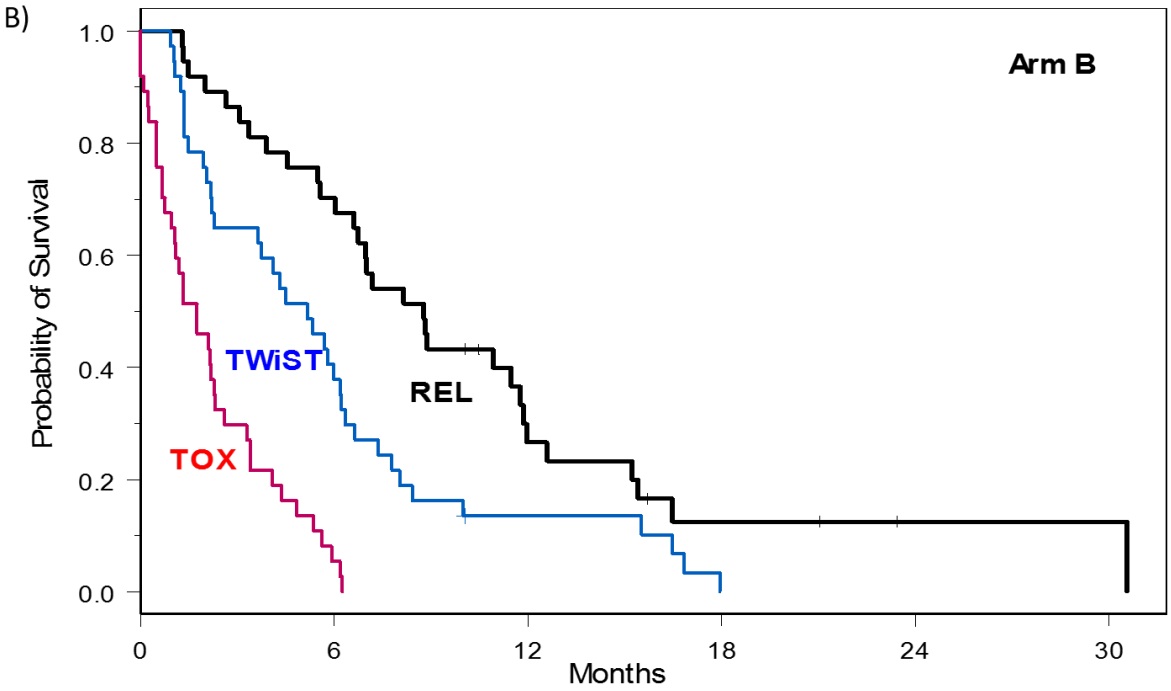

B)
Arm B
Probability of Survival
1.0
0.8
0.6
0.4
0.2
0.0
TWiST
REL
TOX
0
6
12
18
24
30
Months

Figure S4

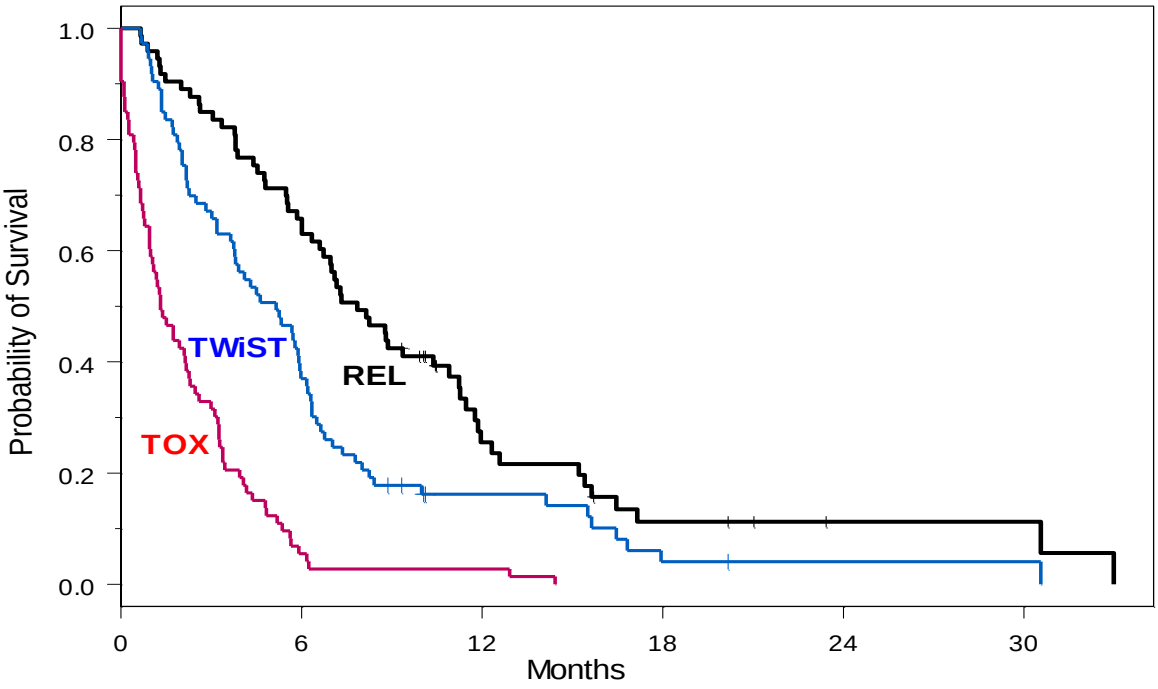

1.0
0.8
0.6
0.4
0.2
0.0
Probability of Survival
TWiST
REL
TOX
0
6
12
18
24
30
Months

Table S1

	Hazard Ratio (HR)	95% CI	P-value
PFS			
(1)Treatment Arm (A vs. B)	0.88	0.54-1.42	0.60
(2)Treatment Arm (A vs. B)	0.86	0.52-1.43	0.56
Age	1.00	0.97-1.02	0.80
OS			
(3)Treatment Arm (A vs. B)	1.12	0.67-1.87	0.68
(4)Treatment Arm (A vs. B)	1.12	0.67-1.88	0.67
Age	1.00	0.97-1.03	0.89

Table S2

Second-line therapy or third-line therapy received by cross over patients after progression	Arm A	Arm B
Gemcitabine and nab-paclitaxel	4	2
FOLFIRINOX	2	2
5FU and gemcitabine	1	2
Gemcitabine and cisplatin	1	2
Gemcitabine and oxaliplatin	1	0
Gemcitabine alone	1	1
FOLFOX	2	2
FOLFIRI	1	0
5FU alone	0	2
Other Clinical Trial	3	0

Table S3

Plasma Biomarker+	DCR p-value	DCR p-value*	PFS p-value*
Fractalkine	**0.124**	**0.18**	**0.026**
IL-6	**0.026**	**0.044**	0.62
IL-8	**0.013**	**0.02**	**0.0499**
RANTES	0.212	0.16	**0.041**
VEGF-A	0.023	0.25	**0.71**

*adjusted for treatment arm

+ Additional plasma biomarkers that were not significantly associated with DCR or PFS in any analysis included the following: IP-10, MCP-1, MIP1a, MIP1b, Gro-a, Eotaxin, SDF-1, IFN-alpha, IFN-gamma, IL-12p70, IL-13, IL-1b, IL-2, IL-4, TNF-alpha, GM-CSF, IL-10, IL-17A, CD40, IL-17F, IL-12p40, MCP-3, MIG, IFN-beta.

Table S4

Cellular Biomarker+	DCR p-value*	PFS p-value*
$CD8^+CD45RA^+$	**0.04**	**0.006**
$CD4^+CD137^+$	0.11	0.65
$CD4^+CD223/LAG3^+$	**0.042**	0.082
% NK cell ($CD3^-CD56^+$)	**0.046**	0.077
% B cell ($CD19^+CD20^+$)	**0.02**	0.059

*adjusted for treatment arm

+ Additional cellular biomarkers that were not significantly associated with DCR or PFS in any analysis include $CD3^+CD56^+$ (NKT cells), $CD3^+CD56^+CD69^+$ (activated NKT cells), HLA-DR^{low} $CD11b^+CD33^+$ (MDSC), $CD4^+CD25^+GITR^+CD127^{low}$ (T regs), $CD4^+CD45RA^+$, $CD8^+CD137^+$, $CD8^+CD223^+$ and $CD4^+$ or $CD8^+$ cells that co-stained with the following CD markers: CD69, CD71, CD95, CD134, CD137, CD152 (CTLA4), CD178, CD272, CD279 (PD1), CD45RO, TIM3.

Table S5.

Restricted Mean in Months (Area under Curve)	Arm A (95% CI)	Arm B (95% CI)	Difference (95% CI)
(1) TOX	2.42 (1.48-3.55)	2.18 (1.58-2.84)	0.24 (-0.88-1.56)
(2) TWiST	5.15 (2.78-7.89)	3.74 (2.51-5.15)	1.41 (-1.38-4.33)
(3) REL	2.38 (0.90-4.09)	4.90 (2.86-6.88)	-2.52 (-5.01-0.23)
(4) PFS	7.57 (4.74-10.67)	5.92 (4.33-7.58)	1.65 (-1.61-5.04)
(5) OS	9.95 (6.80-13.54)	10.82 (7.85-13.93)	-0.87 (-5.26-3.87)

Table S6

Q-TWiST in Months	Arm A (95% CI)	Arm B (95% CI)	Difference (95% CI)
Utox=0, Urel=0	5.15 (2.78-7.89)	3.74 (2.51-5.15)	1.41 (-1.38-4.33)
Utox=0, Urel=0.5	6.34 (3.87-9.14)	6.19 (4.34-8.18)	0.15 (-2.99-3.47)
Utox=0, Urel=1.0	7.53 (4.86-10.69)	8.64 (5.98-11.47)	-1.11 (-4.99-3.13)
Utox=0.5, Urel=0	6.36 (3.78-9.30)	4.83 (3.44-6.33)	1.53 (-1.45-4.63)
Utox=0.5, Urel=0.5	***7.55 (4.90-10.53)***	***7.28 (5.28-9.40)***	***0.27 (-3.12-3.84)***
Utox=0.5, Urel=1.0	8.74 (5.88-12.10)	9.73 (6.93-12.71)	-0.99 (-5.14-3.44)
Utox=1.0, Urel=0	7.57 (4.74-10.67)	5.92 (4.33-7.58)	1.65 (-1.61-5.04)
Utox=1.0, Urel=0.5	8.76 (5.82-12.00)	8.37 (6.17-10.68)	0.39 (-3.30-4.30)
Utox=1.0, Urel=1.0	9.95 (6.80-13.54)	10.82 (7.85-13.93)	-0.87 (-5.26-3.87)

Table S7

Restricted Mean in Months (Area under Curve)	Month (95% CI)
(1) TOX	2.30 (1.74-2.96)
(2) TWiST	4.33 (3.05-5.76)
(3) REL	3.79 (2.49-5.21)
(4) PFS	6.63 (5.08-8.35)
(5) OS	10.42 (8.13-12.76)

Table S8

Q-TWiST in Months	Month (95% CI)
Utox=0, Urel=0	4.33 (3.05-5.76)
Utox=0, Urel=0.5	6.23 (4.68-7.84)
Utox=0, Urel=1.0	8.12 (6.11-10.20)
Utox=0.5, Urel=0	5.48 (4.08-7.02)
Utox=0.5, Urel=0.5	7.38 (5.68-9.12)
Utox=0.5, Urel=1.0	9.27 (7.14-11.46)
Utox=1.0, Urel=0	6.63 (5.08-8.35)
Utox=1.0, Urel=0.5	8.53 (6.66-10.46)
Utox=1.0, Urel=1.0	10.42 (8.13-12.76)