- **Protocol number:** BVD-CNV2.
- **Document title:** Phase 1 safety study of intratumoral injection of *Clostridium novyi*-NT spores in patients with treatment-refractory solid tumor malignancies.
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Phase I safety study of intratumoral injection of *Clostridium novyi*-NT spores in patients with treatment-refractory solid tumor malignancies

Protocol Amendment 2

Sponsor:	BioMed Valley Discoveries, Inc. 4520 Main Street, Suite 1650 Kansas City, MO 64111
Contract Research Organization:	PAREXEL International 200 West Street Waltham, MA 02451
Sponsor Protocol Number:	BVD-CNV2
Development Phase:	Phase 1 (safety, efficacy, spore disposition, immune/inflammatory correlation)
Last Revised:	Final Protocol 10/22/2012 Amendment #1 05/10/2013 Amendment #2 09/02/2014

The clinical study will be conducted according to the protocol and in compliance with Good Clinical Practices (GCP), the Declaration of Helsinki (Version 2008), and with other applicable regulatory requirements.

SIGNATURE PAGE

Declaration of Sponsor or Responsible Medical Expert

Protocol Title: Phase I safety study of intratumoral injection of *Clostridium novyi*-NT spores in patients with treatment-refractory solid tumor malignancies

This clinical study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the study agent, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki (Version 2008), and the guidelines on Good Clinical Practices (GCP) applicable to this clinical study.

Sponsor Signatory/Responsible Medical Expert

Sept 02, 2014

Date

SIGNATURE PAGE

Declaration of the Investigator

Protocol Title: Phase I safety study of intratumoral injection of *Clostridium novyi*-NT spores in patients with treatment-refractory solid tumor malignancies

This clinical study protocol was subjected to critical review and has been released by the Sponsor. The information it contains is consistent with current risk and benefit evaluation of the study agent, as well as with the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki (Version 2008), and the guidelines on Good Clinical Practices (GCP) applicable to this clinical study.

Responsible investigator of the local study center

Name	Date
Title	
Institution	

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List of Abbreviations

Abbreviation	Definition
ABG	Arterial blood gas
ADL	Activities of daily living
AE	Adverse event
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
AUC	Area under the curve
BP	Blood pressure
BSC	Biological safety cabinet
C. novyi-NT	*Clostridium novyi*-Non Toxic
CBC	Complete blood count
CBER	Center for Biologics Evaluation
CEA	Carcinoembryonic antigen
CFR	Code of Federal Regulations
CNS	Central nervous system
COBALT	Combination Bacteriolytic Therapy
CPGT	Cell Processing and Gene Therapy Facility
CRP	C-reactive protein
CT	Computed tomography
CTC	Common Toxicity Criteria
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose-limiting toxicity
DNA	Deoxyribonucleic acid
Dob	Dobutamine
Dop	Dopamine
ECG	Electrocardiogram
Echo	Echocardiogram
ECOG	Eastern Cooperative Oncology Group (performance status scale)
eCRF	Electronic case report form
EDTA	Ethylenediaminetetraacetic acid
Epi	Epinephrine
FDA	Food and Drug Administration
FiO_2	Fraction of inspired oxygen
GCP	Good Clinical Practice
GCS	Glascow Coma Scale
GI	Gastrointestinal
GMP	Good Manufacturing Practice

Abbreviation	Definition
HR	Heart rate
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IND	Investigational New Drug
INR	International normalized ratio
IRB	Institutional Review Board
IV	Intravenous
IT	Intratumoral
K_2	Dipotassium
Lambdaz (λz)	The terminal elimination rate constant determined by selection of at least three data points on the terminal phase of the concentration-time curve.
MAP	Mean arterial pressure
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NaCl	Sodium chloride
NCI	National Cancer Institute
NG	Nasogastric
Norepi	Norepinephrine
NSAID	Non-steroidal anti-inflammatory drug
O_2	Oxygen
PaO_2	Partial pressure of arterial oxygen
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEG	Percutaneous endoscopic gastrostomy
PEG-J	Percutaneous enteral gastric/jejunal
PI	Principal Investigator
PK	Pharmacokinetic(s)
PR	Partial response
PT	Prothrombin time
PTT	Partial thromboplastin time
RECIST	Response Evaluation Criteria in Solid Tumors
ROC	Receiver operating characteristic
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Stable disease
SOFA	Sequential Organ Failure Assessment
SOP	Standard Operating Procedure
SpO_2	Oxygen saturation by pulse oximetry
SST	Serum separator tube

Abbreviation	Definition
STS	Soft tissue sarcoma
$T_{½}$	Terminal elimination half-life calculated as: ln2/Lambdaz
ULN	Upper limit of normal
WBC	White blood cell

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Objectives of the Study

- To determine the safety profile, dose limiting toxicities (DLT), and maximum tolerated dose (MTD) of *Clostridium novyi*–NT (*C. novyi*-NT) in humans with treatment-refractory solid tumor malignancies when administered as a single intratumoral (IT) injection.

- To document preliminary anti-tumor activity of both the injected tumor and an overall response after administering a single IT injection of *C. novyi*-NT in humans with treatment-refractory solid tumor malignancies. The evaluation of anti-tumor activity will include: 1) a response for the injected tumor and 2) an overall response.

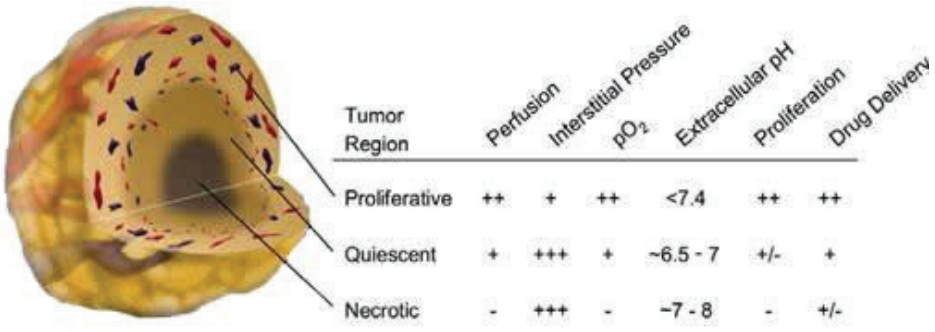
- To study the presence of circulating *C. novyi*-NT spores after administration as a single IT injection to humans with treatment-refractory solid tumor malignancies (see Section 4.1 Study Calendar, Table 4 and Section 8.2 *C. novyi-NT* detection in circulation and/or abscess material/post-dose tumor sample).

- To measure the host immune and inflammatory response to *C. novyi*-NT administered as a single IT injection in humans with treatment-refractory solid tumor malignancies.

1. Introduction and Background

Strategies that successfully target and destroy human cancers exploit differences between normal and malignant tissues[1]. Such differences can be found at the molecular level, as is the case with genetic aberrations, or more holistically, as with the physiological aberrations in a tumor. It is known that malignant solid tumors are usually composed of a necrotic core and a viable rim. Therapeutic interventions to date have focused on the well-vascularized outer shell of the tumor, but few have targeted the inner hypoxic core. The inner core of a tumor has unique characteristics that differentiate it from normal tissues. The core has a poor vascular supply and is therefore deficient in nutrients and oxygen. As a site of active cellular necrosis, the lack of a functional vascular supply limits the clearance of noxious cell breakdown and results in a low pH.

Figure 1: Tumor microenvironment (Jain et al., 2001)[2]



Such an environment is not suitable for growth of most human cells but is an environment favorable to the growth of certain anaerobic bacteria. Over 60 years ago, this concept led investigators to inject spores of *Clostridium histolyticum* into tumor-bearing animals[3]. Remarkably, the bacteria germinated only in the necrotic core of the tumor and liquefied it. In the 1950s and 1960s, spores from *Clostridium butyricum* were injected into patients with a variety of very advanced solid tumor malignancies[4-6]. Many patients had significant germination and destruction of large portions of their tumors, but the very poor health and advanced stage of these patients made their management difficult and the absence of complete clinical responses subdued further pursuit of this approach.

1.1 *Clostridium novyi*-NT

The agent presented for use in humans in this Phase I study is *C. novyi*-NT. It was developed after a large panel of anaerobic bacteria was screened for the ability to germinate and populate in mouse tumor models[1]. The anaerobe *Clostridium novyi* (ATCC #19402) had the best anti-tumor efficacy in this screen. It was subsequently rendered nonpathogenic by selecting a clone without the major toxin (α-toxin) responsible for systemic toxicities associated with this organism[1,7,8]. The resulting clone, named *Clostridium novyi*-NT, where the NT stands for non-toxic, was employed in a series of preclinical studies based on a strategy termed Combination Bacteriolytic Therapy (COBALT) that combined *C. novyi*-NT therapy with traditional chemotherapy or radiation[1,7].

1.2 Preclinical PK of *C. novyi*-NT spores[9]

Pharmacokinetic studies indicate that *C. novyi*-NT spores, when administered intravenously (IV) to mice, are rapidly cleared from the circulation (> 99% spores are cleared within 1 hour) and sequestered

within the reticuloendothelial system. Long-term distribution studies reveal that these spores are eventually eliminated from all tissues by one year. Delivered in spore form (dormant stage), *C. novyi*-NT germinates (transitions from the spore to the vegetative state) when exposed to the hypoxic regions of tumors. Thus, the systemic toxicities of *C. novyi*-NT have been shown to be greater in tumor-bearing than in non-tumor-bearing animals.

1.3 Toxicity Studies of *C. novyi*-NT in Non-Tumor-Bearing Animals[9]

Non-tumor-bearing mice and rabbits showed no clinically apparent signs (morbidity, mortality, or clinical appearance) of toxicity regardless of treatment dose administered intravenously. However, examination of tissues at necropsy revealed both gross and microscopic inflammatory changes that appeared to be treatment-dose dependent. These findings, primarily in the liver, spleen, and adrenals, were noted at doses of 5×10^8 spores/kg or greater. Non-tumor-bearing animals receiving lower doses showed no gross or microscopic abnormalities at necropsy. In animals that received high doses, resolution of inflammation was evident on Day 28 and all signs of inflammation were absent in all animals by 1 year. To determine if *C. novyi*-NT spores would germinate in non-tumor hypoxic tissue, studies in elderly mice with atherosclerotic plaques and experimental myocardial infarctions were treated with *C. novyi*-NT. There was no evidence of spore localization or germination within these vascular lesions. At the conclusion of the study, no clinical or pathologic abnormalities (other than the pre-existing cardiovascular lesions) were noted in these mice. These studies demonstrated that *C. novyi*-NT is an agent with no clinical and minimal pathologic toxicity in non-tumor-bearing animals.

1.4 Efficacy and Toxicity Studies in Tumor-Bearing Animals

Intravenous injection of spores into immune-competent tumor-bearing mice leads to lysis of the tumor and an intense inflammatory response. In mice, one of three outcomes is typically observed: One subset (25 to 35% of mice) are cured (no tumor recurrence after one year of observation) and develop long-term immunity to the original tumor[8]. Another subset (65 to 75%) demonstrates complete clinical responses, but experiences recurrence with regrowth of the original tumor. The remaining subset (0 to 20%, depending on the experiment) demonstrates tumor destruction but develops significant clinical toxicity 2 to 5 days after the initiation of therapy. Relatively simple measures, such as hydration, are adequate to reduce this toxicity, often entirely eliminating it[9]. Studies in larger animals (rabbits) show the same cure and recurrence rates with IV *C. novyi*-NT therapy, but do not show the life-threatening clinical toxicity observed in a subset of mice. Treatment-related death was observed in tumor-bearing mice, but not in rabbits, treated with *C. novyi*-NT spores[9]. In these studies toxicity was related to both spore dose and tumor size and appeared to be at least partially related to dehydration, as it could be effectively managed with supportive care (hydration) and/or antibiotics. In moribund mice, no specific clinical laboratory or pathologic end-organ damage was noted and the only significant finding was reversible hepatosplenomegaly. Cured mice had rare remnant inflammatory changes in the liver and spleen, but were otherwise no different than untreated animals. These studies show that toxicity in tumor-bearing animals can be pronounced (death) in mice with large tumors, but was minimal in larger animals (rabbits), and was manageable in mice with hydration or antibiotics.

1.5 Exploratory Study of Intratumoral Administration of *C. novyi*-NT in Companion Canines Bearing Spontaneous, Naturally Occurring Solid Tumors

C. novyi-NT has been evaluated in an exploratory and open-label study at multiple veterinary oncology centers in companion canines with spontaneous, naturally occurring solid tumors with tumor histology enriched for soft tissue sarcomas (STS). The study was designed to evaluate multiple cycles of intratumoral (IT) injections of *C. novyi*-NT spores in canines. The study has been completed. Findings are summarized as follows.

Study Conduct:
In this study, 16 companion dogs received between one and four treatment cycles of intratumoral (IT) administrations of *C. novyi*-NT spores at 1 x 10^8 spores diluted in 100 µL of saline. Eleven out of 16 dogs received all four treatment cycles. Dogs may have received fewer than four treatment cycles due to tumor ablation (2), toxicity (2), or progressive disease (1). There were 10 males and 6 females with average age of 11 years and average weight of 31 kilograms. The tumor types treated in this study were soft tissue sarcoma (13), osteosarcoma (1), melanoma (1), and mast cell tumor (1). The study follow-up period was three months.

Safety:
Most adverse events were mild in severity, with hyperthermia/fever and tumor inflammation being the most common AEs expected. All fever events were Grade I or II. Tumor inflammation and abscess events, generally considered desired mechanisms of the treatment, were limited to Grades I or II for all dogs but one (who experienced Grade III inflammation).
SAEs were observed in 25% (4/16) of dogs treated; three out of 4 of the events were associated with tumor inflammation that required prolonged hospitalization. It is unclear if the remaining event, cervical steatitis causing neck pain, was associated with *C. novyi*-NT or metastasis given the necropsy findings of a melanoma lesion in the fat of the cervical spine.
When comparing *C. novyi*-NT at 1 x 10^8 spores/dog IT treatment to 1 x 10^8 spores/m^2 IV treatment in the C-100 study, dogs receiving IT treatment experienced far fewer severe AE (Grade III or IV). Twenty-five percent (4/16 dogs) receiving IT treatment experienced severe AE compared to 57% (8/14 dogs) receiving IV administration of *C. novyi*-NT in the C-100 study.

Efficacy:
The incidence of objective response was 38% and included dogs with soft tissue sarcoma and mast cell tumor. Though debriding was performed in 3/6 dogs with objective response, this procedure was undertaken to eliminate necrotic cellular debris and to potentiate the healing of *C. novyi*-NT induced abscesses; complete healing was observed in 2-4 weeks for all dogs whose tumors were debrided. The progression-free rate (at Day 90) was 88%. Four of the eight dogs evaluable at Day 90 had no surgical debridement during the study, including one high grade STS - a tumor type and grade not expected to be progression-free in 90 days without therapy.

1.6 Rationale for Intratumoral Treatment of *C. novyi*-NT in Human Patients

The favorable risk/benefit profile observed from the preliminary results of the ongoing companion canine study with *C. novyi*-NT IT treatment provides a compelling rationale for translating the intratumoral administration of *C. novyi*-NT to a Phase I investigational study in human patients with solid tumors that are either refractory to standard therapy or without an available standard therapy. The Phase I study of intravenous administration of *C. novyi*-NT spores in patients with treatment-refractory solid tumors [NCT01118819] has been closed so that all efforts could be directed to the

intratumoral program. The IT treatment protocol will target patients with superficial tumors measureable, palpable or clearly identifiable under ultrasound or radiographic guidance and amenable to intratumoral injection of *C. novyi*-NT spores.

1.7 Management of Adverse Effects Related to Treatment with *C. novyi*-NT

Local Effects

As highlighted in the ongoing companion canine studies (Section 1.5 Exploratory Study of Intratumoral Administration of *C. novyi-NT* in Companion Canines Bearing Spontaneous, Naturally Occurring Solid Tumors), *C. novyi*-NT germination can occur within tumor tissues following IT dosing, and may lead to tumor inflammation and tumor destruction. Expected inflammatory sequelae may include local erythema, warmth, pain, and tenderness near a tumor injection site. These changes may progress and result in tissue abscessation in the tumor lesion.

Preliminary experience from the ongoing canine study also demonstrates that tumor inflammation can be managed by monitoring and standard medical support measures. Supportive care during the period of time when a localized *C. novyi*-NT infection is being established can result in successful abscess resolution as well as subsequent clinical responses. Similarly, application of antibiotics can be delayed in favor of other interventions including, hydration, analgesia, and abscess lancing and drainage.

Systemic Effects

The current protocol administering *C. novyi*-NT spores via an intratumoral route is expected to result in minimal systemic side effects. However, in order to predict and respond to early signs of systemic toxicity, this study will utilize the SOFA score (Table 1) to guide antibiotic initiation.

Validated scoring strategies exist that can predict the risk of death from systemic insults, including infection. Originally developed for stratifying septic patients, the SOFA scoring system (Sequential Organ Failure Assessment) has been used to predict mortality[10-12]. The SOFA score criteria and scoring system are shown below. Scores can range from 0 to 24 and are calculated from the sum of the six organ systems evaluated (respiratory, coagulation, liver, cardiovascular, central nervous system [CNS], and renal).

Table 1: The SOFA Score (Ferreira et al., 2001.)[10]

Variables	SOFA Score 0	1	2	3	4
Respiratory Pao_2/Fio_2, mm Hg	>400	≤400	≤300	≤200†	≤100†
Coagulation Platelets $\times 10^3/\mu L$‡	>150	≤150	≤100	≤50	≤20
Liver Bilirubin, mg/dL‡	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Cardiovascular Hypotension	No hypotension	Mean arterial pressure <70 mm Hg	Dop ≤5 or dob (any dose)§	Dop >5, epi ≤0.1, or norepi ≤0.1§	Dop >15, epi >0.1, or norepi >0.1§
Central nervous system Glasgow Coma Score Scale	15	13-14	10-12	6-9	<6
Renal Creatinine, mg/dL or urine output, mL/d‖	<1.2	1.2-1.9	2.0-3.4	3.5-4.9 or <500	>5.0 or <200

*Norepi indicates norepinephrine; Dob, dobutamine; Dop, dopamine; Epi, epinephrine; and Fio_2, fraction of inspired oxygen.
†Values are with respiratory support.
‡To convert bilirubin from mg/dL to µmol/L, multiply by 17.1.
§Adrenergic agents administered for at least 1 hour (doses given are in µg/kg per minute).
‖To convert creatinine from mg/dL to µmol/L, multiply by 88.4.

Several studies have shown SOFA scores are good predictors of mortality in the intensive care unit (ICU) setting. Scores at time of admission and the highest score achieved during hospitalization were strong predictors (Figure 2)[10]. Receiver operating characteristic (ROC) curves using admission (initial) and the highest SOFA scores for prediction of mortality repeatedly show area under the curve (AUC) values of between 0.79 and 0.90, respectively (Figure 3)[10]. Sequential organ failure assessment scores determined from the optimal threshold (best cut-off point) to discriminate between survival and non- survival in both cases were greater than 8. With scores above 8 predicting mortality rates averaging 55% (range 26 to 95%) and those below this threshold averaged 13% (range 0 to 21%).

Figure 2: **Mortality rate in relation to the changes in SOFA scores at (A) time of admission or (B) the highest SOFA score achieved during hospitalization. (Ferreira et al., 2001.)[10]**

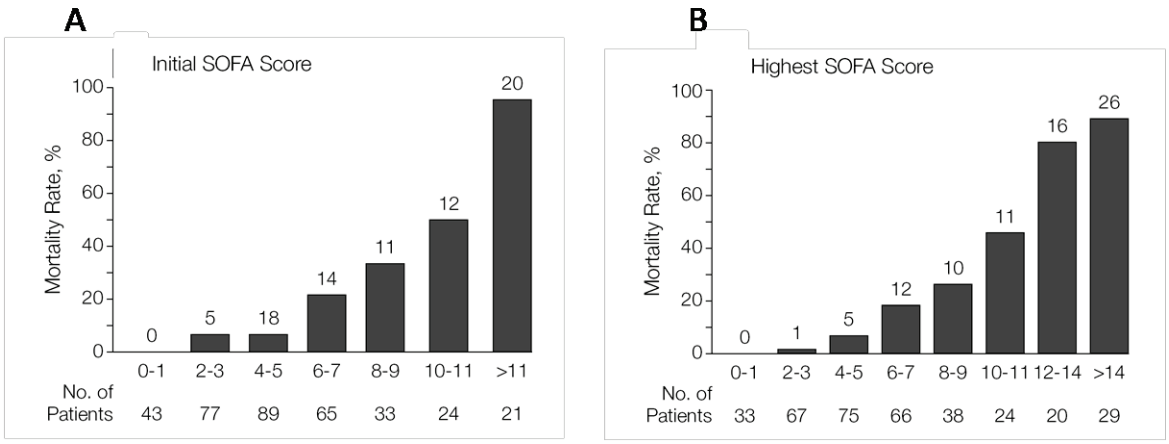
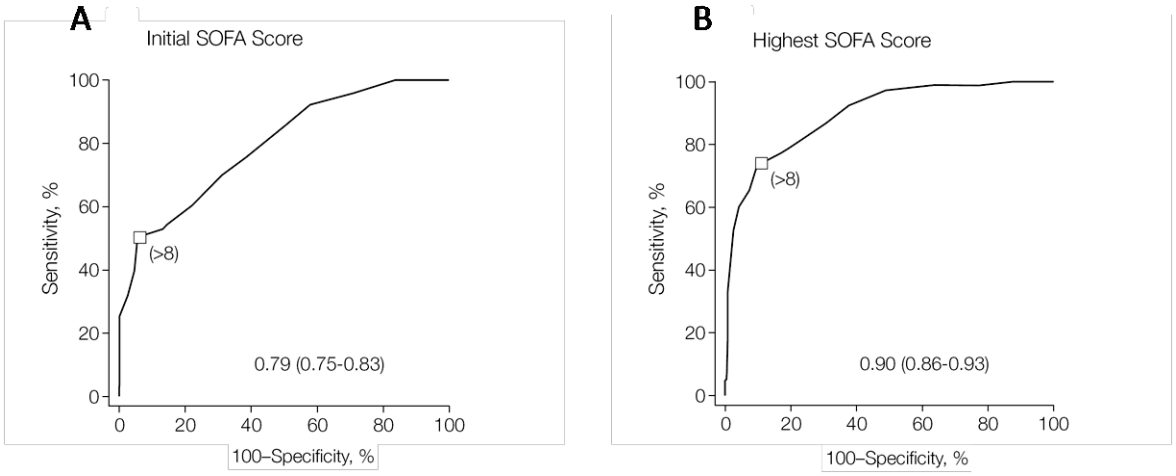


Figure 3: **Comparison of the area under the ROC curves for prediction of mortality using SOFA scores at time of admission or at the highest measured SOFA score achieved during hospitalization. (Ferreira et al., 2001.)[10]**



In addition, when applied to bacteremic patients, SOFA scores at time of bacteremia were significantly lower in the survivor groups who averaged 7.3 as compared with the non-survivors at 10.0 ($p < 0.001$)[13]. The best cut-off SOFA score in this group of patients was calculated to be 7.5 from a ROC curve with an AUC of 0.77.

In this study, we will utilize the SOFA score and temperature to guide antibiotic initiation in our patients treated with *C. novyi*-NT during the in-patient phase. Following administration of *C. novyi*-NT, the patients will be scored at least every 8 hours or when there is a change in status. Although SOFA scores below 7.5 to 8.0 were the derived thresholds to discriminate between survivors and non-survivors, this protocol is designed to start antibiotics in a more conservative manner. Therefore, antibiotics will be initiated when:

a. The total SOFA score is 5 or greater; or
b. The SOFA score in any of the following organ systems reaches 2 (respiratory, coagulation, cardiovascular, CNS); or
c. The SOFA score in either the liver or renal systems reaches 3; or
d. A patient is found to have 2 temperatures > 38.5°C lasting >12 hours beginning 5 days following administration of the study agent; or
e. Initiation of antibiotics is indicated based on the investigator's clinical judgment.

The decision to start antibiotics will be based on the patient's progress, temperature and the SOFA score. Once the decision to start antibiotics is made, parenteral piperacillin/tazobactam and oral or parenteral metronidazole will be administered until the patient is afebrile, clinically stable and has negative blood cultures for *C. novyi*-NT for at least 48 hours. The patient will then receive oral or parental metronidazole for 6 weeks (if the parenteral route is chosen, then a temporary central line will be placed) followed by oral doxycycline 100 mg orally twice per day indefinitely. Alternate options for antibiotic coverage for patients with allergies can be discussed in consultation with the Medical Monitor.

Patients in whom the criteria for starting IV antibiotics are never met during in-hospital phase will still be required to start on therapy with oral doxycycline (100 mg orally twice per day) on Day 7, even in the absence of symptoms attributable to the study agent. Oral doxycycline will be continued indefinitely. Specifically, Day 7 CT/MRI will be reviewed by the PI with his/her study team prior to patient discharge. If CT/MRI scan indicates evidence of abscess formation, the patient will remain hospitalized and will be started on IV antibiotics (see Sec. 5). If no evidence of abscess is identified, the patient will be discharged as planned on PO antibiotics (doxycycline 100 mg orally twice per day indefinitely). Supportive care measures, including antipyretics, analgesics, fluids and pain control, will be managed by the primary inpatient team with guidance from the Principal Investigator (PI) and Medical Monitor. In preclinical studies vigorous intravenous hydration appeared to improve outcomes following *C. novyi*-NT injection in tumor-bearing animals. Need for surgical or percutaneous drainage will be assessed in a case by case basis and appropriate consultation may be requested if clinically indicated.

2. Investigational Plan

2.1 Patient Population

The study population is patients with solid tumor malignancies that are refractory to standard therapy or without available standard therapy. Men and women of all races and ethnic groups are eligible for this trial.

2.2 Eligibility Criteria

All eligibility criteria must be met for patients to be eligible for enrollment. Screening studies will remain valid up to 3 weeks (21 days) prior to treatment.

2.2.1 Inclusion Criteria

1. Diagnosis of an advanced solid tumor malignancy. There must be a target tumor which is measureable, palpable or clearly identifiable under ultrasound or radiographic guidance and amenable to percutaneous injection of *C. novyi*-NT spores. The targeted lesion must have a longest diameter ≥ 1 cm and ≤ 12 cm and be measurable as defined by RECIST 1.1 criteria. The target lesion must not be located in either the thoracic, abdominal or pelvic cavities or in the brain. There must be no clinical, no functional, and no radiographic evidence of bone involvement at the site of the target lesion.
2. History of prior treatment with at least one line of systemic anticancer therapy, when an approved systemic therapy is available, and no curative option is available for continued treatment.
3. At least 4 weeks have elapsed since the completion of major surgery, and the patient has fully recovered from this surgery and any post-surgical complications.
4. Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less (Appendix 5: ECOG Performance Status Scale*).
5. Patient is at least 18 years of age.
6. Patient is capable of giving informed consent.
7. Patient of childbearing potential (defined by the clinical sites' standards) is using adequate birth control measures (e.g., barrier method with spermicide; intrauterine device; implantable or injectable hormonal contraceptives; surgical sterilization) for the duration of the study and will continue to use such precautions for 12 months after receiving treatment.
8. Patient has no significant valvular heart disease (trace or mild valvular stenosis or regurgitation is allowed).
9. Patient is able to stay within 45 minutes driving time of an emergency room for 28 days after dosing.
10. The patient has a caregiver for 28 days after dosing.

2.2.2 Exclusion Criteria

1. Positive pregnancy test.
2. Serum creatinine level ≥ 1.5 x the upper limit of normal (ULN), chronic renal failure requiring hemodialysis or peritoneal dialysis.
3. Patient has any of the following hematologic parameters:
   - Platelet count equal to or less than 100,000/mm^3
   - Hemoglobin less than 9.0 g/dL
   - Absolute neutrophil count (ANC) less than 1,000 /mm^3
4. Oxygen saturation ($Sp0_2$) of less than 95% on room air.
5. Mean arterial blood pressure (BP) of less than 70 mmHg.
6. Glasgow Coma Score (GCS; see Appendix 3: SOFA Score Calculations) of less than 15.
7. Treatment with an investigational drug within the past 30 days or 5 half-lives of that drug, whichever is shorter.
8. Documented primary brain malignancy or brain metastases.
9. Clinically significant ascites or clinical evidence or history of portosystemic hypertension or cirrhosis.
10. Laboratory evidence of hepatic dysfunction indicated by any of the following:
    - Bilirubin ≥ 1.5 x the ULN
    - Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) above 2.5X the ULN

- Alkaline phosphatase above 2.5X the ULN
- International normalized ratio (INR) greater than 1.3

11. Patient has a foreign body which in the opinion of the treating investigator could be difficult to manage in case of infection (e.g. prosthetic hip).
12. Clinically significant pleural effusion.
13. Clinically significant pericardial effusion, circumferential pericardial effusion, or any effusion greater than 1.0 cm at any location around the heart.
14. Need for ongoing treatment with an immunosuppressive agent.
15. History of solid organ transplantation (with the exception of a corneal transplant > 3 months prior to screening).
16. History of an ischemic insult in the previous 12 months (myocardial infarction, cerebral vascular accident, ischemic tissue from injury, transient ischemic attack).
17. History of a significant medical illness deemed by the PI or local investigators as unsuitable for the trial. For example:
    i. Symptomatic congestive heart failure
    ii. Psychiatric Illness/social situation that may make study dangerous
    iii. Unstable angina pectoris
18. Asplenia.
19. Antibiotic allergies that would preclude treatment for a *C. novyi*-NT infection.
20. Treatment with antibiotics within 2 weeks (14 days) of dosing.
21. Active and clinically significant systemic or localized infection.

3. Study Design

Patients who provide informed consent will undergo screening procedures within 21 days prior to *C. novyi*-NT spore administration. Patients will report to the clinical site within 24 hours of Day 0 to reconfirm eligibility and for baseline assessments as described in Table 4. Eligible patients will be admitted and enrolled sequentially into a dosing cohort (see Table 3 and dose escalation details below). Spore administration will occur on Day 0, and patients will remain in-house for observation through Day 7 (a total of 8 days). Patients may be allowed for an extended in-hospital stay for observation if a tumor abscessation and destruction develops in the treated tumor lesion site. Patients will return to the clinical site for follow-up visits at the following time points: Days 11, 14 (Week 2), 18, 21 (Week 3), 25, 28 (Week 4) and 2, 4, 8, and 12 months post-dose. Assessments of safety, efficacy, and disposition of *C. novyi*-NT will be performed at scheduled time points throughout the study (see Study Calendar and Table 4).

A standard "3+3" dose escalation will be used. The following definitions and rules will be employed:

- Toxicity Criteria will be those listed in the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0, which is available at http://ctep.cancer.gov/reporting/ctc.html
- An adverse event (AE) of Grade 3 toxicity lasting three (3) days or longer or Grade 4 toxicity of any duration that is assessed to be at least possibly related to study agent will be considered a DLT. Signs and symptoms of *C. novyi*-NT infection which are associated with a SOFA score > 5 that require invasive intervention including percutaneous drainage of an abscess or that result in prolonged hospitalization (> 5 days) will also qualify as DLTs.
- DLT assessment period is defined as 4 weeks after dosing of the last patient in a cohort.
- Toxicity will be assessed from administration of study agent up to 8 weeks.
- The following dose escalation rules will apply (see Table 2):

Table 2: Dose Escalation Decision Rules

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	The dose escalation will proceed. Three patients will be enrolled at the next dose level
1 out of 3	Three more patients will be enrolled at this dose level • If zero of these three patients experience a DLT, then dose escalation will proceed to the next dose level. • If one or more patients suffer a DLT, then dose escalation will stop. This dose will be the maximally administered dose (i.e., the minimum intolerated dose). Three additional patients will be entered at the next lowest dose level (i.e., the MTD) if only three patients were treated previously at that dose.
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (i.e. the minimum intolerated dose). Three additional patients will be entered at the next lowest dose level (i.e., the MTD) if only three patients were treated previously at that dose.

- Additional patients will be enrolled at the MTD to achieve a total of six patients at that dose level if fewer than six patients were treated previously at that dose.
- Doses will be increased in successive cohorts until the MTD is reached or until complete germination and tumor regression occurs, making higher doses unnecessary.
- The MTD (or dose at which complete germination and tumor regression occurs) will be the recommended dose for future Phase II *C. novyi*-NT IT administration studies.

Patients will be closely monitored by a dedicated team of clinicians for infectious complications or other treatment-emergent adverse events with the objective of deciding if and when to initiate antibiotics and/or additional interventions (Section 5. Management of Study Agent-related Toxicity). Patients will be followed with serial imaging studies for therapeutic response. We will observe the first patient in a cohort for a minimum of 2 weeks before treating the second patient, while there will be no required minimum observation interval before subsequent patients in the same cohort can be dosed. We will observe for a minimum 4-week interval between cohorts. The planned follow-up period for every patient who receives *C. novyi*-NT will be 1 year.

3.1 Dosing

3.1.1 Starting Dose Rationale

The starting dose (Cohort 1) is $1x10^4$ spores administered as a single IT injection. The starting dose is about 700x lower than the Cohort 1 total dose administered in the Phase 1 safety study for intravenous injection of *C. novyi*-NT spores (NCT01118819) in which a starting dose of $1x10^5$ spores/kg, equivalent to a total dose of ~$7x10^6$ spores, was administered to an average 70 kg patient. The starting IT dose of $1x10^4$ spores is 10,000x lower than the IT study in companion dogs (Section 1.5 Exploratory Study of Intratumoral Administration of *C. novyi-NT* in Companion Canines Bearing Spontaneous, Naturally Occurring Solid Tumors).

3.1.2 Dose Escalation Scheme

Three to six patients will receive IT treatment with *C. novyi*-NT at a dose corresponding to assigned cohort (Table 3), starting with Cohort 1. *C. novyi*-NT spores will be administered as an IT injection into the target lesion. Radiographic guidance may be used at the investigator's discretion to aid in targeting an area of necrosis in the target lesion. Patients will be given IV hydration after dosing.

Table 3: *C. novyi*-NT Spores Planned Dose Levels for Intratumoral Injection

Cohort	Number of Patients	Dose ($\times 10^4$ spores)
1	3 to 6	1
2	3 to 6	3
3	3 to 6	10
4	3 to 6	30
5	3 to 6	100

3.1.3 Investigational Product and Injection

The investigational product, *C. novyi*-NT spores, are packaged in vials and stored in a refrigerator with temperature maintained between 2 – 8°C. The concentration of spores is [redacted] suspended in sterile phosphate buffer saline (PBS). The actual concentration may vary slightly among the GMP lots.

The investigational drug preparation will occur on the day of administration to the patient. The dilution of concentrated spore suspension will be performed at the time of administration in normal sterile saline infusion bags of appropriate size to achieve the required dose based on dose level in each respective cohort. The injectable volume will be 3 mL and will be withdrawn from the saline bag with an appropriate size of syringe (3cc or 5cc) and needle (22-24 gauge). The injection of 3 mL diluted spore suspension may be injected with the needle or alternate injection device and the injection can be redirected to multiple distinct sites in the tumor to achieve adequate dispersal of spores throughout the tumor. The injection may be carried out under radiographic guidance.

3.2 Concomitant Medications

Any medicinal product, prescribed or over-the-counter, including herbal and other non-traditional remedies, is considered a concomitant medication. Prior and concomitant medication use will be recorded for the 4 weeks prior to screening until the final study visit. Antibiotic usage is prohibited during the 14 days prior to study agent administration (Day 0).

Concomitant medications will be used for the management of adverse effects related to treatment with *C. novyi*-NT spores and for the treatment of unrelated AEs.

Treatment with antibiotics (parenteral piperacillin/tazobactam and metronidazole; oral or parenteral metronidazole; oral doxycycline) may be needed. Please refer to Section 5. Management of Study Agent-related Toxicity for full details.

3.3 Patient Discontinuation

A discontinuation occurs when an enrolled patient ceases participation in the study, regardless of the circumstances, prior to completion of the protocol. The investigator must determine the primary reason for discontinuation. An attempt will be made by site staff to have all subjects who discontinue prematurely from the study complete, at a minimum, the procedures done at the final visit (i.e., the 12 Month visit).

A patient will be discontinued from study if any of the following occur:

- The investigator withdraws the patient from the study in agreement with the Sponsor and Medical Monitor.
- The patient withdraws consent at any time after agent administration. Every effort will be made to determine why any subject withdraws from the study prematurely.
- The patient is lost to follow-up. A genuine effort must be made to determine the reason(s) why a patient fails to return for the necessary visits. If the subject is unreachable by telephone, a registered letter, at the minimum, should be sent requesting him/her to contact the clinic.
- Disease progression in the injected tumor site based on RECIST 1.1 criteria, as assessed by the investigator and not prior to 8 weeks post-dosing. (Caution should be taken in assessing progression in the injected tumor prior to 8 weeks since *C. novyi*-NT germination may cause inflammatory changes resulting in “pseudoprogression”.)
- The patient starts a new anti-cancer therapy or enrolls in another interventional clinical trial.

After discontinuation, further radiographic assessment will not be required and SAEs will no longer be reported, unless they are assessed as related or possibly related to *C. novyi*-NT treatment by the PI. Every effort will be made to collect survival information even after discontinuation, including phone calls on a quarterly basis for 12 months to the patient, patient’s relatives or primary oncologist.

All patients who withdraw prematurely prior to 4 weeks after dosing for reasons other than an AE will be replaced before dose escalation. At any time during the study, the Sponsor and Investigators may request that cohorts should be enlarged. Such requests will be discussed with the Sponsor and the Medical Monitor, and should be based on all data existing at that time.

3.4 Early Stopping Rule

Based on the literature, the median survival of patients with treatment-refractory advanced metastatic solid tumor malignancies is 6 months. These patients will be treated with an intratumoral injection of the study agent, and we do not expect study agent-related mortality. The sponsor may terminate the trial if toxicity observed indicates an unfavorable risk-to-benefit ratio.

4. Study Procedures

Potentially eligible patients will be referred to the clinical site staff for study screening by contacting an investigator or the research nurse for this study. Consent will be administered by an investigator prior to all study specific screening procedures. The inclusion/exclusion criteria will be reviewed to determine if the patient qualifies for the study. Once the patient is deemed eligible, a date for study agent administration will be determined. Patients must begin treatment within 21 days of screening.

4.1 Study Calendar

Pre-treatment (Screening)	Informed Consent; Medical and Medication History, Physical Exam; Vital signs (BP, HR, respiratory rate, oral body temperature); SpO_2; ECOG; Routine lab work * (including serum pregnancy); Research lab work**; Blood sampling for phosphorus, PT/PTT, uric acid, magnesium, and CRP; CT/MRI Scans; Brain MRI; Echo; ECG; weight collection; begin AE monitoring
Day of admission and treatment (Day 0)	<u>Pre-dose (day -1 - 0):</u> Medical history, Physical exam; SpO_2; ECOG; Blood Type (follow unit standards); ECG; Routine lab work* (including serum pregnancy); Cultures ***; Research lab work**; Blood sampling for phosphorus, PT/PTT, uric acid, magnesium, and CRP; Pre-dose tumor biopsy (injected lesion) for immunological assessment and tissue banking, Vital signs; SOFA; weight collection; continue AE and concomitant medication monitoring. Study agent will be administered as described in Section 2. Investigational Plan and Appendix 1: Study Agent Information. <u>Post-dose (relative to IT injection):</u> • Continuous SpO_2 monitoring; • Vital signs every 15 minutes (+/- 5 minutes) for 60 minutes, then every 30 minutes (+/- 10 minutes) for 60 minutes, then every 60 minutes (+/- 15 minutes) for 120 minutes; Subsequent vital sign checks will be performed every 4 hours (+/- 1 hour); • Routine lab work at 60 minutes (+/- 15 minutes) and 12 hours post-dose (+/- 2 hours); • Research lab work at 60 minutes (+/- 15 minutes) and 12 hours post-dose (+/- 2 hours); • Blood sampling for phosphorus, PT/PTT, uric acid, magnesium, and CRP at 60 minutes (+/- 15 minutes) and 12 hours post-dose (+/- 2 hours); • Anaerobic blood cultures for *C. novyi*-NT at 60 minutes (+/- 15 minutes) and 12 hours post-dose (+/- 2 hours); • Abscess material post-dose for culture, immunological assessment and banking, when performed; • SOFA at 8 and 16 hours post-dose (+/- 1 hour)****; • Continue AE and concomitant medication monitoring; • IV hydration after dosing (IV fluids and duration are at PI's discretion).
Days 1-7	Vital signs every 4 hours (+/- 1 hour) beginning at 24 hours post-dose; Continuous SpO_2 monitoring; SOFA every 8 hours (+/- 1 hour) beginning at 24 hours post-dose****; Physical once daily; ECOG once daily; Routine lab work* once daily beginning at 24 hours post-dose; Blood sampling to measure phosphorus, PT/PTT, uric acid, magnesium, and CRP once daily; Research lab work** once daily; Anaerobic blood cultures for *C. novyi*-NT once daily; CT/MRI Scans (Day 7 only); Weight collection (Day 7 only); Continue AE and concomitant medication monitoring; Abscess material for culture, immunological assessment and banking (when performed). Patients will be discharged after all procedures/assessments are completed on Day 7.
Outpatient Visits [Abscess material post-dose for culture, immunological assessment and banking (when performed)]	
Day 11, Day 18, Day 25	Physical; vital signs; ECOG, Continue AE and concomitant medication monitoring
Day 14, Day 21	Physical; Vital signs; ECOG; Routine lab work*; Research lab work**; Blood sampling to measure phosphorus, PT/PTT, uric acid, magnesium, and CRP; Weight collection; Anaerobic blood cultures for *C. novyi*-NT. Continue AE and concomitant medication monitoring;

Day 28, Months 2, 4, 8, and 12	Physical; Vital signs; ECOG; Routine lab work*; Research lab work **; Blood sampling to measure phosphorus, PT/PTT, uric acid, magnesium, and CRP; Weight collection; Anaerobic cultures for *C. novyi*-NT; CT/MRI Scans; Continue AE and concomitant medication monitoring. Tumor biopsy from injected lesion and/or non-injected lesion at month 1 (optional) and month 2 (required) post-dose for immunological assessment, and tissue banking. Biopsy collection and time points may vary depending on tissue availability, local management of tumor inflammation or abscessation, and whether the biopsy can be easily and safely obtained. Biopsy for cultures may be performed at PI's discretion.

BP = blood pressure; HR = heart rate; SpO_2 = oxygen saturation (by pulse oximetry); ECOG = Eastern Cooperative Oncology Group Performance Status Scale; CT = computed tomography; MRI = magnetic resonance imaging; Echo = echocardiogram; ECG = electrocardiogram; AE = adverse event; SOFA = Sequential Organ Failure Assessment; PT = prothrombin time; PTT = partial thromboplastin time; CRP = C-reactive protein

*Routine lab work includes: complete blood count (CBC) with differential and serum chemistry panel

**Research lab work includes whole blood and serum for studies described in Section 8. Correlative Studies.

***Blood cultures (anaerobic for *C. novyi*-NT and aerobic).

****Clinical laboratory values for SOFA assessments will be based on the most recent values obtained for clinical laboratory tests.

Table 4: Schedule of Assessments

Admit Procedure	Screen (Day -21 to -1)	(Day -1 to 0)	Dosing (Day 0)	Day 0- Post-dose Time Point (Relative to IT Injection)												Days 1 to 7* (24 h to 192 h)	Follow-up Day 11, 18, 25 (±1 day)	Follow-up Day 14, 21 (± 1 day)	Follow-up Wk 4 to 12 Mon** (± 2 days)
				15 min	30 min	45 min	60 min	90 min	2 h	3 h	4 h	8 h	12 h	16 h	20 h				
Eligibility Evaluation																			
Informed Consent	X																		
Inclusion/Exclusion	X																		
Pregnancy Test	X	X																	
Medical History	X	X																	
Brain MRI	X																		
Echocardiogram	X																		
ECG	X	X																	
Safety Evaluation																			
Physical Exam	X	X														X	X	X	X
Vital Signs[e]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X[a]	X	X	X
SpO_2	X	X	X	→	→	→	→	→	→	→	→	→	→	→	→	→			
SOFA Score Asses		X										X		X		X[b]			
ECOG	X	X														X	X	X	X
CBC with differential	X	X					X						X			X[c]		X	X
Serum Chemistry panel[f]	X	X					X						X			X[d]		X	X
Magnesium	X	X					X						X			X		X	X
Phosphorus	X	X					X						X			X		X	X
Uric Acid	X	X					X						X			X		X	X
PT/PTT	X	X					X						X			X		X	X
CRP	X	X					X						X			X		X	X
Weight collection	X	X														X (D7)		X	X
AE Monitoring	X	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Concomitant Medications	X	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Aerobic blood cultures[1]	X	X																	
Anaerobic blood cultures (for detection of C. novyi-NT)		X					X						X			X		X	X
Blood type		X																	
Efficacy Evaluation																			
CT or MRI scans[2]	X															X (D7)			X
Serum Banking	X	X					X						X			X		X	X
Whole blood Research[g]		X														X (D1 & D7)		X	X
Tumor biopsy [h]		X[i]																	x[i]
Abscess material[j] .				→	→	→	→	→	→	→	→	→	→	→					→
Treatment																			
C. novyi-NT			X																
IV hydration			X[k]																

ECOG = Eastern Cooperative Oncology Group (Performance Status Scale); SpO_2 = oxygen saturation (by pulse oximetry); SOFA = Sequential Organ Failure Assessment; CBC = complete blood count; PT = prothrombin time; PTT = partial thromboplastin time; CRP = C-reactive protein; ECG = electrocardiogram; AE = adverse event; CT = computed tomography; MRI = magnetic resonance image

* Procedure will be performed either once daily or at the specified time points.

** Visits will be at 28 days and 2, 4, 8, and 12 months (±2 days) after dosing.

1. Additional aerobic and anaerobic blood cultures may also be performed when clinically indicated.
2. Whichever modality best suited for tumor assessment. Same imaging modality should be used throughout the course of the study. CT/MRI scans for targeted injection lesion and any metastatic lesions.

a. Vital signs were obtained every 4 hours beginning 24 hours post-dose (i.e., at 24, 28, 32, 36, 40, 44, 48…192 h).
b. SOFA with SpO_2 will be conducted every 8 hours beginning 24 hours post-dose (i.e., at 24, 32, 40, 48…192 h).
c. Blood will be collected for CBC with differential once daily beginning 24 hours post-dose.
d. Blood will be collected for serum chemistry panel once daily beginning 24 hours post-dose.
e. Vital signs will include blood pressure, heart rate, respiratory rate, and oral temperature.
f. In addition to magnesium, phosphorus, uric acid (measured at specified time points), serum chemistry panel will include: glucose, calcium, albumin, total protein, sodium, potassium, lactate dehydrogenase, chloride, blood urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and bilirubin.
g. Whole blood for immunological assessment is collected at Day-1 to 0, D7, D14, D21, week 4, and month 2 (there is no D1 collection); whole blood for banking is collected at Day -1 to 0, D1, D7, D14, D21, week 4 to 12 month follow-up visits, and will be used for *C. novyi*-NT detection and immunological assessment.
h. Tumor biopsy collections will be done only to those patients who have given consent for tumor biopsy collections and will be used for immunological assessment and tissue banking.
i. Pre-dose tumor biopsy is collected from the injected lesion; Post-dose tumor biopsy (injected and/or non-injected lesions) at month 1 (optional) and month 2 (required) may depend on tissue availability, local management of tumor inflammation or abscessation, and whether the biopsy can be easily and safely obtained. Biopsy for cultures may also be performed at PI's discretion.
j. Abscess material samples collected and banked only when performed as part of the management of local tumor inflammation and abscessation.
k. IV hydration after dosing. IV fluids and duration are at PI's discretion.

4.2 Safety Evaluation

Monitoring for AEs will occur from the time of informed consent until the patient completes his/her participation in the study. The post-dosing observation period will begin with an 8-day inpatient stay (Days 0 to 7). This inpatient stay can be extended and requires that at least 2 days elapse from the resolution of any signs of infection or serious AEs (SAEs; defined in Section 6. Adverse Event Reporting below) before discharge.

During the inpatient observation period, the patient will be assessed for safety per the schedule of events. Sequential organ failure assessment scoring will be performed every 8 hours or with any change in clinical status. Any clinical signs or symptoms of infection will be reported within 1 hour of onset by the inpatient clinical team to the local investigator or delegates. Management of study agent related toxicity will be governed by the criteria set-forth in the following Section 5. Management of Study Agent-related Toxicity.

Following the inpatient observation period and during the first 28 days post-treatment, patients will be followed in two ways; all patients will be required to be evaluated during scheduled visits on Days 11, 14, 18, 21, 25 and 28. Patients will receive daily phone interviews on the days when they are not scheduled for a clinic visit to evaluate and catalogue any changes in status. These phone interviews will consist of a review of symptoms of infection (see Appendix 4: Symptoms of Infection), temperature checks and compliance with antibiotic regimen. At any time, the PI or local investigator will be available for immediate consultation by phone (information provided on Patient ID card), and if necessary in person at the outpatient clinic or as an inpatient based on the clinical scenario. The patient must have a caregiver available through Day 28, and must stay within 45 minutes driving time of an emergency room for 28 days after dosing. The PI or local investigator will be available 24/7 throughout the entire study.

After the first Day 28 visit, the patient will continue to be evaluated at scheduled visits, and the PI or local investigator will remain available as needed.

During all scheduled visits, a physical examination, vital signs and ECOG status will be assessed. Clinical laboratory tests, and blood cultures will be obtained during the visits outlined in the schedule of events. Imaging will be performed, as scheduled to investigate the possibility of abscess formation or to explore the possibility of unexpected toxicity. Additional consultants from other subspecialties will be called upon as the clinical situation dictates.

4.3 Safety Evaluation Summary

Days 0 to 7	Inpatient observation	
Days 8 to 28	Twice weekly scheduled visits	Daily phone interviews
Months 2 to 12	Scheduled visits	

5. Management of Study Agent-related Toxicity

Management decisions regarding all day-to-day or emergent matters will be performed by the inpatient clinical team. The PI and Medical Monitor may be consulted regarding management decisions.

After spore injection and during the initial 8 days, supportive care measures, including antipyretics, analgesics, fluids, and pain control, may be managed by the primary inpatient team with guidance from the PI or treating investigator.

Initiation of antibiotics will be based on assessments by the investigator and the inpatient clinical team. The guiding criteria for initiating antibiotics will be as follows:

1. A total SOFA score of 5 or greater; OR
2. An individual organ system SOFA score of 2 or greater within the respiratory, cardiac, coagulation, or central nervous systems; OR
3. An individual organ system SOFA score of 3 or greater within the liver or renal systems; OR
4. Antibiotics will be initiated if a patient is found to have 2 temperatures >38.5°C lasting >12 hours beginning 5 days following administration of the study agent; OR
5. Initiation of antibiotics is indicated based on the investigator's clinical judgment and regardless of the SOFA score.

Sequential Organ Failure Assessment score calculation is described in detail in Appendix 3: SOFA Score Calculations.

Patients in whom the criteria for starting IV antibiotics are never met during in-hospital phase will still be required to start on therapy with oral doxycycline (100 mg orally twice per day) on Day 7, even in the absence of symptoms attributable to the study agent. Oral doxycycline will be continued indefinitely. Specifically, Day 7 CT/MRI will be reviewed by the PI with his/her study team prior to patient discharge. If CT/MRI scan indicates evidence of abscess formation, the patient will remain hospitalized and will be started on IV antibiotics (see Sec. 5). If no evidence of abscess is identified, the patient will be discharged as planned on PO antibiotics (doxycycline 100 mg orally twice per day indefinitely).

Antibiotics will be administered according to the following schedule:

1. Parenteral piperacillin/tazobactam and metronidazole until the patient is afebrile and clinically stable for 48 hours, switch to
2. Six weeks of oral or parenteral metronidazole. If the parenteral route is chosen, then a temporary central line will be placed after terms in #1 are met, followed by
3. Indefinite oral doxycycline (100 mg twice daily)

Substitution for any antibiotics can be made for patients with allergy or if intolerance or hypersensitivity develops. Choice of replacement antibiotic will be based on the sensitivity profile of *C. novyi*-NT, which will be provided by the Sponsor to the clinical site(s), and input from Infectious Diseases as appropriate.

Need for surgical drainage will be assessed on a case-by-case basis, and surgical consultation will be requested as the case dictates. When surgical drainage of the tumor is conducted a material sample will

be collected for culture and banking.

6. Adverse Event Reporting

6.1 Definitions

An Adverse Event (AE) is any new untoward medical occurrence or worsening of a pre-existing medical condition in a clinical investigation subject administered an investigational medicinal product, even if it does not have a causal relationship with that product. Any unfavorable or unintended sign, symptom, or disease temporally associated with the use of the study agent, whether or not it is considered study agent-related will be considered an AE. This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of the study agent. An unexpected AE is any AE that is not explicitly described in the Investigational New Drug (IND) application, protocol, or informed consent document.

All Grade 3 and 4 clinical laboratory results that represent an increase in severity from baseline will be reported as AEs. A Grade 1 or 2 clinical laboratory abnormality should be reported as an AE only if it is considered clinically significant by the investigator.

A Serious Adverse Event (SAE) is any AE, occurring at any dose and regardless of causality that:

- Results in death.
- Is life-threatening. Life-threatening means that the patient is at immediate risk of death from the reaction as it occurs; it does not include a reaction which hypothetically might cause death had it occurred in a more severe form.
- Requires inpatient hospitalization or prolongation of pre-existing hospitalization except when an extended inpatient hospitalization is required to manage local tumor inflammation or abscessation in the injected tumor lesion site. This does not include scheduled admissions for study-related issues (e.g., inpatient IT injection of study agent)
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly/birth defect
- Is an important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization, but may be considered an SAE when, based upon appropriate medical judgment, may jeopardize the patient and may require an intervention (medical or surgical) to prevent one of the outcomes listed above as a definition of SAE (e.g., bronchospasm).

Clarification of the terms 'severe' and 'serious' should be made. These terms are not synonymous. 'Severe' is a measure of intensity and may describe a non-serious AE. Intensity is graded as mild, moderate, or severe. 'Serious' is a term that characterizes events that pose a threat to a patient's life or important bodily function.

6.2 Reporting

All AEs, including observed or volunteered problems, complaints, or symptoms from the time of informed consent until 8 weeks post dose are to be recorded, regardless of causality. After 8 weeks post-dose, only AEs with possible, probable, or definite relationship to the study agent will be reported with follow up as noted above for AEs within the first 8 weeks post treatment.

Adverse events will be evaluated against the baseline history and physical examination and intensity graded using the NCI CTCAE, Version 4.0, which can be accessed at http://ctep.cancer.gov/reporting/ctc.html. Clinical syndromes associated with laboratory abnormalities are to be recorded as appropriate (e.g., diabetes mellitus). When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

When recorded the AE is to be evaluated for:

1. *Duration*: Time of onset and length of time of toxicity.

2. *Intensity* (NCI CTCAE 4.0):

Grade 1:	Mild or asymptomatic or mild symptoms or clinical or diagnostic observations only or intervention not indicated.
Grade 2:	Moderate or minimal, local or noninvasive intervention indicated or limiting age-appropriate instrumental ADL*.
Grade 3:	Severe or medically significant but not immediately life-threatening or
Grade 4:	Life-threatening consequences or urgent intervention indicated.
Grade 5:	Death related to AE.

ADL = activities of daily living; AE = adverse event
*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications,
and not bedridden.

3. *Causal relationship* between the study agent and the AE will be based on:

    - temporal relationship of the event to the study agent;
    - whether an alternative etiology has been identified;
    - biological plausibility.

The relationship to treatment will be determined by the investigator and reported on the SAE form, as appropriate. The following terms will be used:

- Not Related: likely or clearly due to causes other than the study drug
- Related: possibly, probably, or definitely related to the study drug

The action taken (none, study drug withheld permanently, medication given, or other), outcome

(resolved, ongoing, death, or unknown), and whether the event was serious will also be recorded.

6.3 Pregnancy

The Sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical study.

Pregnancy alone is not regarded as an AE unless there is a suspicion that the study agent may have interfered with the effectiveness of a contraceptive medication. Elective abortions without complications should not be regarded as AEs, unless they were therapeutic abortions (see below). Hospitalization for normal delivery of a healthy newborn should not be considered an SAE.

Each pregnancy must be reported by the Investigator to the Sponsor within 30 days after becoming aware of the pregnancy. The Investigator must follow-up and document the course and the outcome of all pregnancies even if the patient withdraws from the clinical study or if the clinical study is finished. All outcomes of pregnancy must be reported by the Investigator to the Sponsor on the pregnancy outcome report form within 30 days after he/she has gained knowledge of the normal delivery or elective abortion.

Any SAE that occurs during pregnancy must be recorded on the SAE report form (e.g., maternal serious complications, therapeutic abortion, spontaneous abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, birth defect) and reported in accordance with the procedure for reporting SAEs.

6.4 Follow-up of Adverse Events

All AEs reported for a patient, irrespective of the suspected causality, will be monitored until the event has resolved or stabilized, until any abnormal laboratory values have returned to baseline or stabilized at a level acceptable to the PI or investigator and Medical Monitor, until there is a satisfactory explanation for the changes observed, or until the patient is lost to follow-up.

6.5 Serious Adverse Event Reporting

The local investigator will review each SAE and evaluate the intensity and the causal relationship of the event to study agent. All SAEs will be recorded from signing of informed consent until 8 weeks after study agent administration. After 8 weeks post-dose, only SAEs with possible, probable, or definite relationship to the study agent will be reported.

Serious AEs must be reported as soon as possible after the PI or investigator has become aware of its occurrence by submitting a completed SAE form to PAREXEL PPD by fax at PPD or via email at PPD

In the event that the site is unable to complete the SAE form to report the event within 24 hours of their becoming aware of the event, the investigators must report the SAE over the telephone via the SAE

answering service at CCI and then provide the completed SAE form via fax or email.

As a minimum requirement, the initial notification should provide the following information:

- Study number
- Subject number
- Gender
- Date of birth
- Name of investigator and full clinical site address
- SAE term
- Details of SAE
- Criterion for classification as 'serious'
- Study drug name, or code if unblinded, and treatment start date
- Date of SAE onset
- Causality assessment (If insufficient information is available to make this classification, then, by default, the SAE will be considered 'related'.)

PAREXEL PPD will notify BioMed Valley Discoveries, Inc. via email of an SAE within 1 business day of discovery or notification.

Initial reports of SAEs must be followed later with detailed descriptions of the events. All relevant information will be recorded and faxed to the PAREXEL PPD within 24 hours of receipt of the information. The Sponsor may also request additional information on the SAE, which the investigator or an authorized delegate must fax to the Sponsor.

Serious AEs will be reported to the following regulatory bodies as described. In addition, an expedited report will be provided that describes any patient who requires tumor drainage or develops sepsis.

6.5.1 Food & Drug Administration (FDA)

In compliance with 21CFR 312.32 the Sponsor will notify the FDA, Center for Biologics Evaluation and Research (CBER) division, of any adverse experience associated with the use of the study agent that is both serious and unexpected. Each notification shall be made as soon as possible and no later than 15 calendar days after the Sponsor's initial receipt of the information. In addition, the Sponsor shall also notify the FDA of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days after the Sponsor's initial receipt of the information. Follow-up information to the initial event report shall be submitted as soon as subsequent relevant information is available.

6.5.2 Institutional Review Board (IRB)

All SAEs will be reported to the IRB per institutional guidelines. If an AE requires modification of the study protocol and informed consent, these modifications will be provided to the IRB with the report of the AE. Follow-up information to the initial event report shall be submitted as soon as subsequent relevant information is available.

7. Efficacy Evaluation

Response and progression will be evaluated using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee (version 1.1: http://www.eortc.be/recist/).

Objective responses will be measured by serial CT or MRI scans of the injected tumor and sites of metastatic involvement. Overall response, based on CT/MRI scan results, will be based on observation of measurable and non-measurable disease as compared to baseline and nadir in target and non-target tumors per RECIST 1.1. CT or MRI scans may be submitted to a third party vendor for review.

8. Correlative Studies

Correlative samples will be collected and stored for up to 5 years. Whole blood samples will be collected for immunological assessments and *C. novyi*-NT detection. When performed, post-injection site abscess material samples will be collected and stored (for up to 5 years). Archived tissue may be used for correlative studies provided that informed consent from the subject is obtained, or when not possible, a waiver of consent is granted by the IRB. Comprehensive information on sample acquisition, handling and storage are to be found in the study manual. Sample tube labels should include the patient identification number/protocol code, sample number and visit number and will be detailed in the study laboratory manual. Refer to study manual for samples storage conditions at the study center until shipment under appropriate conditions to the third party laboratory vendor and/or sample storage location. Samples will be used to evaluate the following parameters:

8.1 Measurement of the anti-*C. novyi*-NT immune and inflammatory responses

The immune and systemic inflammatory responses during *C. novyi*-NT treatment will be evaluated by serial measurement of anti-*C. novyi*-NT antibodies, CRP, selected cytokines, and analysis of selected immune cell populations in blood. C-reactive protein and certain cytokines (IL-6, IL-8, TNF-alpha, etc.) are markers of systemic inflammation. Patient whole blood and serum samples will be obtained as indicated on the study calendar and, when performed, pre-dose and post-dose tumor samples and abscess material will be collected.

8.2 *C. novyi*-NT detection in circulation and/or abscess material/post-dose tumor sample

Scheduled whole blood sampling will take place as indicated in the study calendar (Section 4.1 Study

Calendar) and Table 4. Anaerobic blood cultures and/or abscess material/post-dose tumor sample (when performed) will be assessed to determine the presence (from circulation) of *C. novyi*-NT.

9. Statistical Considerations

Before database lock, a statistical analysis plan (SAP) will be issued as a separate document, providing detailed methods for the analyses outlined below. Any deviations from the planned analyses will be described and justified in the final integrated clinical study report.

Markers of systemic inflammation, such as CRP and cytokines, will be graphed as continuous variables over time, and descriptive statistics will be provided for these variables. Exploratory graphs will be constructed to show the relationship between these responses and their time course.

Individual patient safety data will be listed. Continuous safety data will be summarized by dose level using descriptive statistics, and categorical safety data will be summarized by dose level using frequency tables. Shift tables and categorical frequency tables will be presented for safety data as appropriate.

10. Ethical, Legal, and Administrative Requirements

10.1 Data Recording, Management, and Monitoring

The investigator(s) will prepare and maintain adequate and accurate records of all observations and other data pertinent to the clinical study for each study participant. Frequent communication between the clinical site(s) and the Sponsor and/or their representative is essential to ensure that the safety of the study is monitored adequately. The investigator(s) will make all appropriate safety assessments on an ongoing basis. The Medical Monitor may review safety information as it becomes available throughout the study.

All aspects of the study will be carefully monitored with respect to GCP and standard operating procedures (SOPs) for compliance with applicable government regulations by PAREXEL International. The study monitor will have access to all records necessary to ensure integrity of the data and will review the progress of the study with the PI/local investigator.

10.2 Access to Source Data/Documents

Data collection for this protocol will be accomplished using electronic data capture.

The local investigator will ensure the accuracy, completeness, and timeliness of the data reported to the Sponsor. Data collection processes and procedures will be reviewed and validated to ensure completeness, accuracy, reliability, and consistency. A complete audit trail will be maintained of all data changes. The investigator or designee will cooperate with the Sponsor's representative(s) for the periodic review of study documents to ensure the accuracy and completeness of the data capture system at each scheduled monitoring visit.

The investigator or designee will prepare and maintain adequate and accurate study documents (medical records, ECGs, AE and concomitant medication reporting, raw data collection forms, etc.) designed to record all observations and other pertinent data for each patient receiving study agent.

The investigator will allow contract designees, authorized regulatory authority inspectors, and the IRB to have direct access to all documents pertaining to the study.

Electronic consistency checks and manual review will be used to identify any errors or inconsistencies in the data. This information will be provided to the respective study sites by means of electronic or manual queries.

10.3 Archiving Study Documents

According to International Conference on Harmonization (ICH) guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the study agent. However, these documents should be retained for a longer period if required by the applicable legal and/or regulatory requirements. The Sponsor should inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial-related records are no longer needed.

10.4 Good Clinical Practice

The clinical study also will be carried out in keeping with national and local legal requirements (in accordance with United States Code of Federal Regulations [21 CFR 312.50 and 21 CFR 56]) and abiding by the guidelines of the ICH guidelines on GCP and the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki (Version 2008).

10.5 Protocol Approval and Amendment(s)

Before the start of the clinical study, the clinical study protocol and other relevant documents will be approved by the IRB, in accordance with local legal requirements. All ethical and legal requirements must be met before the first patient is enrolled in the clinical study.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, which must be released by the responsible staff and receive IRB approval prior to implementation (as appropriate).

Administrative changes may be made without the need for a formal amendment, but will also be mentioned in the integrated clinical study report. All amendments will be distributed to all study protocol recipients with appropriate instructions.

11. Subject Confidentiality and Rights

11.1 Patient Information and Informed Consent

Before each patient is enrolled in the clinical study, written informed consent will be obtained according to the regulatory and legal requirements of the participating country. As part of this procedure, the investigator must explain orally and in writing the nature, duration, and purpose of the study, and the action of the study agent in such a manner that the patient is aware of the potential risks, inconveniences, or AEs that may occur. The patient should be informed that he/she is free to withdraw from the study at any time. He/She will receive all information that is required by federal regulations and ICH guidelines.

The informed consent document must be signed and dated; one copy will be handed to the patient, and the investigator will retain a copy as part of the clinical study records. The investigator will not undertake any investigation specifically required for the clinical study until written consent has been obtained. The terms of the consent and when it was obtained must also be documented.

If a protocol amendment is required, then the informed consent document may need to be revised to reflect the changes to the protocol. If the informed consent document is revised, it must be reviewed and approved by the responsible IRB, and signed by all patients subsequently enrolled in the clinical study as well as those currently enrolled in the clinical study.

Separate consent will be requested to obtain/retain blood, serum and abscess material/biopsy samples (when available). Samples will be stored for 5 years for future analysis, which may include genomic evaluation of the tumor tissue, as warranted by our rapidly-advancing understanding in this field. Separate consent will also be requested to obtain, when possible, photographs of the tumor pre- and at various time points post-dose.

11.2 Patient Confidentiality

In order to maintain patient privacy, all electronic case report forms (eCRFs), source documents, study agent accountability records, study reports and communications will identify the patient by initials and the assigned patient number. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by applicable laws and regulations.

11.3 Publication Policy

By signing the clinical study protocol, the PI/local investigator agrees with the use of results of the clinical study for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. If necessary, the competent authorities will be notified of the PI's/local investigator's name, address, qualifications, and extent of involvement.

An investigator shall not publish any data (poster, abstract, paper, etc.) without having consulted with the Sponsor in advance.

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List of Appendices

Appendix 1: Study Agent Information

Study Agent Development/Purification

C. novyi-NT is a gram-positive obligate anaerobe rendered nonpathogenic through selection of a clone in which the bacteriophage carrying the major systemic toxin (α-toxin) gene is absent. The α-toxin is the major toxin responsible for pathogenicity of this organism, and was eliminated by dilution cloning coupled with heat treatment to inactivate extracellular phage. A resultant clone, termed *C. novyi*-NT, was shown not to carry the α-toxin and subsequently used for all pre-clinical work and for Good Manufacturing Practice (GMP) grade agent development for use in humans.

Packaging/Labeling

Omnia Biologics, Inc. is responsible for the manufacturing and for the formulation of *C. novyi*-NT spores. The *C. novyi*-NT spores are packaged in glass vials at a concentration of approximately [redacted] suspended in sterile PBS and will be labeled with the following information: identity of test article, number of particles contained and volume, directions for use, storage requirements, caution statement, lot number.

Storage and Security Conditions

Vials of *C. novyi*-NT spores will be stored at 2-8°C controlled temperature environment under constant temperature monitoring with access limited to authorized study personnel.

Study Agent Preparation

The preparation of the injectable drug suspension will take place on the day of IT administration. The vial(s) containing *C. novyi*-NT spores will be diluted using the appropriate volume of sterile 0.9% sodium chloride (NaCl). *C. novyi*-NT spore preparation and dilution will take place in a designated biological safety cabinet (BSC) and may be carried out at room temperature. The vial should be vortexed to ensure an even suspension of spores before the appropriate volume of aliquot is drawn for dilution. The appropriate volume of spores will be drawn from the vial and added into an appropriate size of sterile saline infusion bag for dilution. The resulting saline bag will be inverted multiple times to ensure that the spores are mixed completely with the saline and there are no visible particulates. 3 mL of the diluted spore suspension will be withdrawn from the saline bag with an appropriate size of syringe (3 mL or 5 mL) for injection. The proper volume of the concentrated spores and the proper size of the saline bag for dilution will be determined by the dose level for respective cohorts, and the exact information will be included in the Investigational Drug Manual.

Study Agent Administration

The study agent will be administered starting with Cohort 1 at a dose of 1×10^4 spores followed by dose escalation as described in Section 3. Study Design. The patient will be given IV hydration after dosing. IV fluids and duration are at PI's discretion.

Study Agent Disposal

In the event of a spill of the study agent, a clean-up protocol exists (Appendix 2: Study Agent Spill Procedure). This protocol and the supplies necessary will be on hand at the time of study agent administration. Otherwise, dosing administration supplies will be collected and sealed in a biohazard bag and destroyed. The vial(s) and dilution bag will be destroyed per the institution's standard for biohazard waste.

Appendix 2: Study Agent Spill Procedure

Procedure is based on the evaluation of the disinfection of *C. novyi*-NT study. If there is a spill of *C. novyi*-NT spores, perform the following:

1. If applicable, promptly remove any contaminated garments
2. If gloves were contaminated, remove gloves and put on new gloves
3. Cover spill with paper towels
4. Flood the spill with 10% bleach
5. After 40 minutes, clean up the spill with paper towels
6. Dispose of the materials in a biohazard bag
7. Wipe the area/equipment with sterile 70% Isopropyl Alcohol (IPA)-saturated towels
8. Dispose of the towels in a biohazard bag
9. Document the spill per clinical site SOPs

Appendix 3: SOFA Score Calculations

Sequential Organ Failure Assessment score will be calculated by adding the individual scores from each organ system based on the table below.

	Modified SOFA Score				
	0	1	2	3	4
Respiration PaO_2/FiO_2 (torr)	>400	≤400	≤300	≤200 With respiratory support	≤100 With respiratory support
SpO_2/FiO_2 (torr)	≥512	<512	<357	<214 With respiratory support	<89 With respiratory support
Coagulation[a] Platelets ($x10^3/mm^3$)	>150	≤150	≤100	≤50	≤20
Liver[a] Bilirubin (mg/dL) (µmol/L)	<1.2 <20	1.2-1.9 20-32	2.0-5.9 33-101	6.0-11.9 102-204	>12.0 >204
Cardiovascular Hypotension	No hypotension	MAP <70 mmHg	Dopamine ≤5 or dobutamine (any dose)[b]	Dopamine >5 or epi ≤0.1 or norepi ≤0.1 or phenylephrine ≤300[c] or vasopressin <0.04 units/min	Dopamine >15 or epi >0.1 or norepi >0.1 or phenylephrine >300[c] or vasopressin ≥0.04 units/min
Central Nervous System (Glasgow coma score scale)	15	13-14	10-12	6-9	<6
	Modified SOFA Score				
	0	1	2	3	4
Respiration PaO_2/FiO_2 (torr)	>400	≤400	≤300	≤200 With respiratory support	≤100 With respiratory support
Renal[a] Creatinine (mg/dL) (µmol/L) or urine output	<1.2 <110	1.2-1.9 110-170	2.0-3.4 171-299	3.5-4.9 300-440 or <500 mL/day	>5.0 >440 or <200 mL/day

Adapted from Vincent JL, de Mendonca A, Cantraine F, Moreno R, Takala J, Suter PM, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med 1998;26(11):1793-800.

PaO_2 = partial pressure of arterial oxygen; FiO_2 = fraction of inspired oxygen = SpO_2: oxygen saturation by pulse oximetry; epi = epinephrine; norepi = norepinephrine; MAP = mean arterial pressure.

a Clinical laboratory values for SOFA assessments will be based on the most recent values obtained for clinical laboratory tests

b Data on file.

c Adrenergic agents administered for at least 1 hour (doses given are in µg/kg/min).

*To convert torr to kPa, multiply the value by 0.1333.

_____ + _____ + _____ + _____ + _____ + _____ = _____

Respiratory Renal Coagulation Liver Cardiovascular CNS (GCS) SOFA Score

Coagulation, Liver, Cardiovascular and Renal scores will be determined from the most recent laboratory values, vital signs, or clinical measurements. Central nervous system evaluations will be assessed using the GCS; the GCS is presented below. The $PaO_2/FI0_2$ ratio for the Respiratory score will be determined using transcutaneous pulse oximetry values to estimate PaO_2 – unless actual measured PaO_2 is available. A conversion chart is shown below.

Some laboratory or clinical parameters may be transient, correctable, spurious, or the result of non- study agent effects (e.g. narcotics, sleeping aids, or anti-emetics on cognitive function). If platelet counts, bilirubin, or creatinine levels are considered as such, then they must be rechecked within 12 hours of the value in question for SOFA scoring purposes. Respiratory, BP, or cognitive parameters should be corrected within 2 hours of the measurement in question. If a non-study agent is clearly responsible for alteration in a specific laboratory or clinical parameter, then this should be documented in the patient's chart and not used for SOFA calculations.

Glasgow Coma Scale (GCS):

The scale comprises three tests: eye, verbal, and motor responses. The three values separately as well as their sum are considered. The lowest possible GCS (the sum) is 3 (deep coma or death), while the highest is 15.

Best eye response (E):

There are 4 grades starting with the most severe:

1. No eye opening
2. Eye opening in response to pain (Patient responds to pressure on the patient's fingernail bed; if this does not elicit a response, supraorbital and sternal pressure or rub may be used)
3. Eye opening to speech (Not to be confused with an awaking of a sleeping person; such patients receive a score of 4, not 3)
4. Eyes opening spontaneously

Best verbal response (V):

There are 5 grades starting with the most severe:

1. No verbal response
2. Incomprehensible sounds (Moaning but no words)
3. Inappropriate words (Random or exclamatory articulated speech, but no conversational exchange)
4. Confused (The patient responds to questions coherently but there is some disorientation and confusion)
5. Oriented (Patient responds coherently and appropriately to questions such as the patient's name and age, where they are and why, the year, month, etc.)

Best motor response (M):

There are 6 grades starting with the most severe:

1. No motor response
2. Extension to pain (adduction of arm, internal rotation of shoulder, pronation of forearm, extension of wrist, decerebrate response)

3. Abnormal flexion to pain (adduction of arm, internal rotation of shoulder, pronation of forearm, flexion of wrist, decorticate response)
4. Flexion/Withdrawal to pain (flexion of elbow, supination of forearm, flexion of wrist when supra-orbital pressure applied; pulls part of body away when nailbed pinched)
5. Localizes to pain (Purposeful movements towards painful stimuli; e.g., hand crosses mid-line and gets above clavicle when supra-orbital pressure applied.)
6. Obeys commands (The patient does simple things as asked.)

The SpO_2/FIO_2 or PaO_2/FIO_2 ratio:

The PaO_2/FiO_2 ratio can be determined from an arterial blood gas (ABG) or estimated from a SpO_2 measurement obtained from a saturation monitor. It is important to note that SpO_2/FiO_2 ratios have not been validated for SpO_2 values greater than 97%. Thus, for patients receiving supplemental oxygen (more than room air [O_2 in room air = 21%]) the site staff will need to either reduce the inspired oxygen briefly to allow a reading to be determined below 97% or perform an ABG to obtain a PaO_2. (For patients who have a SpO_2 value of > 97% on room air, the site can use the PaO_2 conversion value since the inspired oxygen cannot be further manipulated, and any SpO_2 value > 98% while receiving room air will yield a converted SpO_2/FiO_2 of > 512). SpO_2 measurements below 90% warrant a blood gas evaluation.

SpO_2 (%)	PaO_2 (mmHg)
99.7	463
99	171
98	112
97	91
96	81
95	74
94	69
93	65
92	62
91	59
90	57

The fraction of inspired oxygen $FI0_2$ used to calculate the SpO_2/FiO_2 or $PaO_2/FI0_2$ ratio will be based on the values from the following chart.

O_2 (L/Min) by nasal cannula	**FIO_2**
None	0.21
1	0.24
2	0.27
3	0.30
4	0.33
5	0.36
6	0.39

Appendix 4: Symptoms of Infection

The following questions will be asked as part of daily phone interviews on Days 8 to 28:

1. Do you have any fevers? If so, what is your temperature?
2. Do you have any sweats or shaking chills?
3. Do you have any changes in the tumor site (visual, palpable, etc.)?
4. Do you have a fast heartbeat or any palpitations?
5. Do you have any shortness of breath or rapid breathing?
6. Do you have a productive cough?
7. Do you have any nausea or vomiting?
8. Do you have any abdominal pain?
9. Do you have any diarrhea?
10. Do you have any bleeding problems?
11. Do you have any pain with urination?
12. Do you have any changes in urine output?
13. Do you have any lightheadedness or dizziness?
14. Do you have any problems with confusion (noted by you or others)?
15. Do you have a new rash around the tumor site or other areas?

Appendix 5: ECOG Performance Status Scale*

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Courtesy of the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

Appendix 6: Amendment 2 List of Changes

Amendment rationale

The primary rationale for this amendment is to update eligibility criteria, to include additional sample collections for immunological assessment and *C. novyi*-NT detection, and to tighten up logistics issues which had been apparent during the beginning months of the study. Minor grammatical and formatting changes were also made to the protocol for clarity (not listed below).

Administrative changes

- Amendment 2 List of Changes was added as Appendix 6;
- Table of Content was updated to reflect the change; and
- BioMed Valley Discoveries, Inc. contact information was updated.

Modifications to Protocol:
All modifications to the protocol are noted below using the following formatting conventions:

- ~~**Bold and strike through text**~~**:** Deleted Text
- ***Bold and italicized text:*** Added Text

"Objectives of the Study"
The third objective has been modified

Changed text:

To study the ~~**disposition**~~ ***presence*** of circulating *C. novyi*-NT spores after administration as a single IT injection to humans with treatment-refractory solid tumor malignancies (see Section 4.1 Study Calendar, Table 4 and Section 8.2 *C. novyi*-NT detection in circulation and/or abscess material/post-dose tumor sample).

Rationale:
'Presence' is a better terminology since the assays for detection of *C. novyi*-NT spores are not likely to be adequate to document the route of elimination.

Section "1.1 *Clostridium novyi*-NT"
First paragraph has been modified.

Changed text:
The agent presented for use in humans in this ***P***~~**p**~~hase I study is *C. novyi*-NT. It was developed after a large panel of anaerobic bacteria was screened for the ability to germinate and populate in mouse tumor models[1]. The anaerobe *Clostridium novyi* (ATCC #19402) had the best anti-tumor efficacy in this screen. It was subsequently rendered nonpathogenic by selecting a clone without the major toxin (α-toxin) responsible for systemic toxicities associated with this organism[1,7,8]. The resulting clone, named *Clostridium novyi*-NT, ***where the NT stands for non-toxic,*** was employed in a series of preclinical studies based on a strategy termed Combination Bacteriolytic Therapy (COBALT) that combined *C. novyi*-NT therapy with traditional chemotherapy or radiation[1,7].

Rationale: To define NT for clarity.

Section "1.2 Preclinical PK of *C. novyi*-NT spores[9]

Changed text:

Pharmacokinetic studies indicate that *C. novyi*-NT spores, when administered intravenously (IV) ***to mice***, are rapidly cleared from the circulation (> 99% spores are cleared within 1 hour) and sequestered within the reticuloendothelial system. Long-term distribution studies reveal that these spores are eventually eliminated from all tissues by one year. Delivered in spore form (dormant stage), *C. novyi*-NT germinates (transitions from the spore to the vegetative state) when exposed to the hypoxic regions of tumors. Thus, the systemic toxicities of *C. novyi*-NT have been shown to be greater in tumor-bearing than in non-tumor-bearing animals.

Rationale: To provide clarity on the species in which the information was obtained.

Section "1.5 Exploratory Study of Intratumoral Administration of *C. novyi*-NT in Companion Canines Bearing Spontaneous, Naturally Occurring Solid Tumors"

Changed text:

C. novyi-NT ~~**is being**~~ ***has been*** evaluated in an ~~**ongoing**~~ exploratory and open-label study at multiple veterinary oncology centers in companion canines with spontaneous, naturally occurring solid tumors with tumor histology enriched for soft tissue sarcomas (STS). The study was designed to evaluate multiple cycles of intratumoral (IT) ~~**or intravenous (IV)**~~ injections of *C. novyi*-NT spores in canines. ***The study has been completed. Findings are summarized as follows.***

Study Conduct:

In this study, 16 companion dogs received between one and four treatment cycles of intratumoral (IT) administrations of C. novyi-NT spores at 1 x 10^8 spores diluted in 100 µL of saline. Eleven out of 16 dogs received all four treatment cycles. Dogs may have received fewer than four treatment cycles due to tumor ablation (2), toxicity (2), or progressive disease (1). There were 10 males and 6 females with average age of 11 years and average weight of 31 kilograms. The tumor types treated in this study were soft tissue sarcoma (13), osteosarcoma (1), melanoma (1), and mast cell tumor (1). The study follow-up period was three months.

Safety:

Most adverse events were mild in severity, with hyperthermia/fever and tumor inflammation being the most common AEs expected. All fever events were Grade I or II. Tumor inflammation and abscess events, generally considered desired mechanisms of the treatment, were limited to Grades I or II for all dogs but one (who experienced Grade III inflammation).

SAEs were observed in 25% (4/16) of dogs treated; three out of 4 of the events were associated with tumor inflammation that required prolonged hospitalization. It is unclear if the remaining event, cervical steatitis causing neck pain, was associated with C. novyi-NT or metastasis given the necropsy

findings of a melanoma lesion in the fat of the cervical spine.

When comparing C. novyi-NT at 1 x 10^8 spores/dog IT treatment to 1 x 10^8 spores/m^2 IV treatment in the C-100 study, dogs receiving IT treatment experienced far fewer severe AE (Grade III or IV). Twenty-five percent (4/16 dogs) receiving IT treatment experienced severe AE compared to 57% (8/14 dogs) receiving IV administration of C. novyi-NT in the C-100 study.

Efficacy:

The incidence of objective response was 38% and included dogs with soft tissue sarcoma and mast cell tumor. Though debriding was performed in 3/6 dogs with objective response, this procedure was undertaken to eliminate necrotic cellular debris and to potentiate the healing of C. novyi-NT induced abscesses; complete healing was observed in 2-4 weeks for all dogs whose tumors were debrided. The progression-free rate (at Day 90) was 88%. Four of the eight dogs evaluable at Day 90 had no surgical debridement during the study, including one high grade STS - a tumor type and grade not expected to be progression-free in 90 days without therapy.

~~The first cohort of canine subjects were treated with IT administration of *C. novyi* NT at a dose of 1x10^8 spores injected into a single, target tumor per treatment cycle. The study allows for a maximum of 4 cycles of therapy. A cycle of therapy is defined as a single IT injection of *C. novyi* NT into one target tumor. Treated subjects are followed for 90 days following the first exposure to *C. novyi* NT. Subjects may be followed long-term for progression and survival.~~

~~Sixteen canines received IT therapy (Table 1 and Table 2). The study revealed that objective disease response can occur following germination of *C. novyi* NT spores in tumors dosed via IT injection into a single tumor. Germination is evidenced by erythema, warmth and tenderness in and around the injection site. In a subset of cases, these tumor reactions progress and lead to the formation of tissue abscesses and concurrent tumor destruction. Furthermore, robust germination appears to occur in the absence of severe systemic adverse effects.~~

~~1.5.1 Study Objectives and Key Eligibility Criteria in Companion Animals~~

~~The objectives of the study include 1) safety evaluation of IT administration of C. *novyi* NT spores at a dose of 1 x 10^8 spores per injection per cycle for up to 4 cycles of weekly dosing; and 2) anti-tumor efficacy evaluation per RECIST 1.1 guidelines.~~

~~Notable inclusion criteria limit eligibility based on tumor size (1 to 7 cm in the longest diameter) and tumor location (amenable to intratumoral injection). Cases are excluded if they have received antibiotics within 7 days of enrollment or if they have received systemic or local anti-cancer therapy within 21 days of enrollment.~~

~~Enrolled cases can be treated with up to 4 cycles of IT administration of *C. novyi* NT spores at a weekly interval, or at an interval determined jointly by the study Investigators, Medical Monitor and Sponsor based on observed adverse reactions and/or efficacy following completion of each cycle.~~

~~1.5.2 Preliminary Study Results in Companion Animals~~

~~Findings reported here reflect data collected as of Oct 15th 2012. The study is currently open for~~

~~screening additional cases for enrollment.~~

~~Study Demographics and Progress~~
~~Of 16 cases screened, 12 were assigned to receive up to 4 cycles of intratumoral *C. novyi* NT therapy. The demographics are summarized in Table 1.~~

~~Table 1: Demographic Summary of Companion Canines Treated with IT Administration of *C. novyi* NT~~

~~ID~~	~~Breed~~	~~Age~~	~~Sex~~	~~Tumor Histology~~	~~Grade~~	~~# of cycles of IT Doses Received~~
[illegible]	~~Golden Retriever~~	[illegible]	~~M~~	~~Soft tissue sarcoma~~	~~Low~~	[illegible]
[illegible]	~~Golden Retriever~~	[illegible]	~~M~~	~~Soft tissue sarcoma~~	~~Low~~	[illegible]
[illegible]	~~Boxer~~	[illegible]	~~M~~	~~Soft tissue sarcoma~~	~~Low~~	[illegible]
~~04-R04~~	~~Saint Bernard mix~~	~~11~~	~~F~~	~~Chondroblastic osteosarcoma~~	~~High~~	~~1~~
[illegible]	~~Shetland~~	[illegible]	~~M~~	~~Soft tissue sarcoma~~	~~Intermediat~~	[illegible]
[illegible]	~~Golden Retriever~~	[illegible]	~~M~~	~~Melanoma~~	~~High~~	[illegible]
[illegible]	~~Maltese~~	[illegible]	~~M~~	~~Soft tissue sarcoma~~	~~Intermediat~~	[illegible]
[illegible]	~~Labrador Retriever~~	[illegible]	~~F~~	~~Soft tissue sarcoma~~	~~Intermediat~~	[illegible]
[illegible]	~~Husky mix~~	[illegible]	~~M~~	~~Soft tissue sarcoma~~	~~Low~~	[illegible]
[illegible]	~~Labrador Retriever~~	[illegible]	~~M~~	~~Soft tissue sarcoma~~	~~Low~~	[illegible]
[illegible]	~~Shepherd mix~~	[illegible]	~~F~~	~~Soft tissue sarcoma~~	~~Low~~	[illegible]
[illegible]	~~Labrador mix~~	[illegible]	~~M~~	~~Soft tissue sarcoma~~	~~Intermediat~~	[illegible]

~~Preliminary Safety Evaluation in Companion Animals~~
~~An intratumoral dose of 1 x 10^8 *C. novyi* NT spores was well tolerated in cases receiving 1, 2, 3 or 4 cycles of therapy. The adverse events (AEs) were almost exclusively limited to Grade 1 and Grade 2, and most were consistent with the anticipated tumor inflammatory reactions resulting from the mechanism of action of the *C. novyi* NT therapeutic. The AEs were managed effectively following clinical management with hydration, NSAIDs, and tumor debridement.~~

~~Grade 3 AEs were noted in two cases. In one case (#16-R03), Grade 3 diarrhea, tumor swelling and lameness occurred within 4 days following the second cycle of therapy. All symptoms were stable and resolved over the subsequent 7 days following effective management with hydration, NSAIDs, and tumor debridement. The second case (#10-R01) experienced Grade 3 tetraparesis starting 2 days following the second cycle of therapy. The symptoms resolved with antibiotics and corticosteroids, and the subject regained full strength over the subsequent 10 days.~~

~~A Grade 4 AE was noted in one case (#11-R01). Thrombocytopenia (Grade 4) was identified at the Day 90 visit. Symptoms resolved 21 days after the Day 90 visit without any medical treatment. Notably, this subject also exhibited Grade 1 and Grade 3 symptoms of thrombocytopenia at screening and baseline, respectively.~~

~~Preliminary Efficacy Evaluation in Companion Animals~~
~~As of October 15th 2012, 4 of 12 subjects have been evaluated with a partial response (PR), 5 of 12 subjects have been evaluated with stable disease (SD), and 3 of 12 subjects have been evaluated with~~

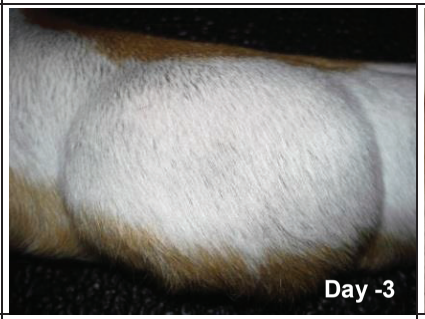
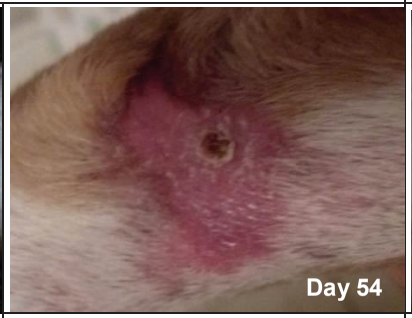
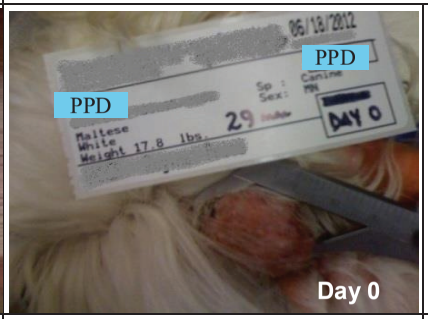
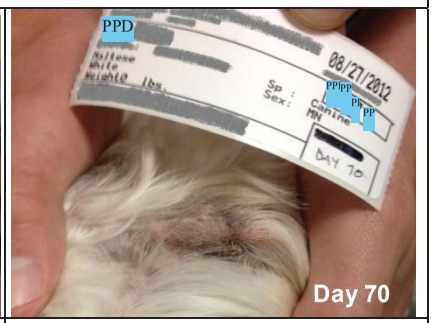
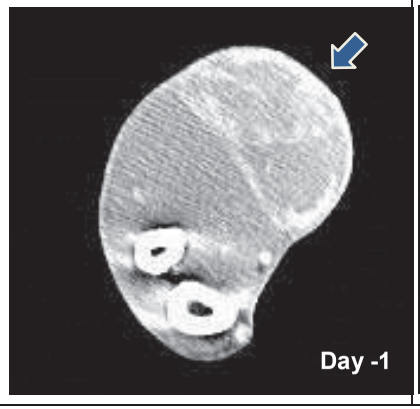
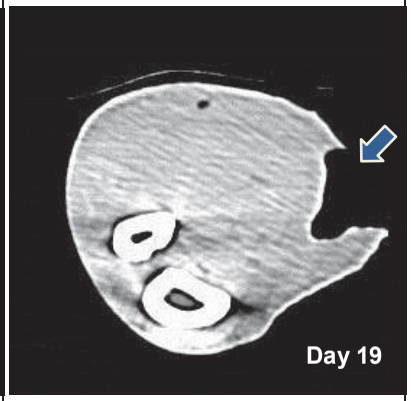
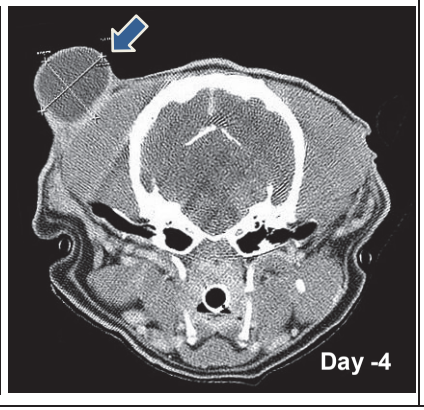
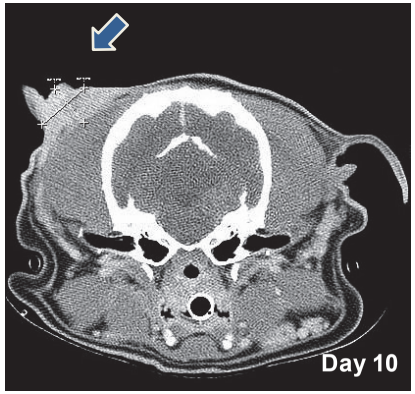
~~progressive disease. A preliminary objective response rate of 33% (4 of 12 subjects) to-date has been observed; these cases are summarized in Table 2. Complete response is difficult to ascertain in the short term given changes to tissue via remodeling that may be occurring at the treatment site post abscessation. CT imaging and other modalities may better distinguish healthy versus tumor tissue at the tumor site.~~

~~In those cases with partial response, the tumor site exuded necrotic or liquefied tissue fr~~agments after ~~limited debridement and drainage. Two of the responders (04-R03 and 11-R01) were assessed to be grossly tumor free at their most recent evaluation at day 60 and day 120, respectively. Subject #04-R03 has no visible remnants of tumor tissue on examination. Subject #11-R01 appears to be tumor free with only remnants of scar tissue on evaluation. Their clinical courses are summarized in Table 3.~~

~~Table 2: Preliminary Evaluation of Cases Treated with *C. novyi* NT IT Therapy with Partial Response (PR) and Stable Disease (SD)~~

~~Case #~~	~~Tumor Measurement (mm, longest diameter)~~		~~Preliminary Evaluation~~
	~~Baseline~~	~~Clinical Course~~	
~~04-R01~~	~~15~~	~~0 (Day 60)~~	~~PR~~
~~04-R02~~	~~46~~	~~52 (Day 21); 73 (Day 25); 0 (tumor fell off) (Day 26)~~	~~PR~~
~~04-R03~~	~~56~~	~~0 (Day 18); 0 (Day 60)~~	~~PR~~
~~11-R01~~	~~29~~	~~9.4 (Day 21); 8.3 (Day 111); suspected scar tissue remnants (Day 120)~~	~~PR~~
~~11-R02~~	~~43~~	~~48 (Day 30)~~	~~SD~~
~~11-R04~~	~~29~~	~~26 (Day 14)~~	~~SD~~
~~16-R02~~	~~91~~	~~100 (Day 32)~~	~~SD~~
~~16-R03~~	~~34~~	~~29 (Day 19)~~	~~SD~~
~~26-R01~~	~~24~~	~~25 (Day 25)~~	~~SD~~

Table 3: Response of Two Cases Treated with *C. novyi*-NT Intratumoral Therapy

Case Features	Case 1 (04-R03, PPD)	Case 2 (11-R01, PPD)
Tumor Type	Peripheral nerve sheath tumor, grade I	Peripheral nerve sheath tumor, grade II
Tumor Size	55.8mm (longest diameter)	29.0mm (longest diameter)
Prior Treatment	None	Surgical excision 10 months prior to enrollment, local tumor recurrence
IT Dose & # Cycles	$1x10^8$ spores/injection; 3 cycles	$1x10^8$ spores/injection; 1 cycle
Clinical Course	Day 15: Moderate infection signs; Grade 1 AEs: lameness/swelling/ bleeding/fever/anorexia; Grade 2 AEs: lethargy and pain. All resolved by Day 19 Day 16: Tumor lanced and debrided Days 16-54: Wound managed with bandage changes, prior tumor site nearly healed	Day 2: Mild infection signs (swelling and erythema) Day 3: Tumor lanced and drained Day 14: Wound healed Day 70: Suspected scar tissue remaining at the tumor site
Biological Activity	Symptoms of tumor destruction starting on Day 14 after the 3rd cycle	Symptoms of tumor destruction starting on Day 2 after 1st cycle
Tumor Gross Response	 	 
CT Scan (Pre- and Post-Treatment)	 	 
Preliminary Outcome	Grossly tumor free at 60 days post 1st cycle	Grossly tumor free at 120 days post 1st cycle

Rationale: To provide final findings of the completed canine study.

Section "1.6 Rationale for Intratumoral Treatment of *C. novyi*-NT in Human Patients"
First paragraph has been modified.

Changed text:

The favorable risk/benefit profile observed from the preliminary results of the ongoing companion canine study with *C. novyi*-NT IT treatment provides a compelling rationale for translating the intratumoral administration of *C. novyi*-NT to a Phase I investigational study in human patients with solid tumors that are either refractory to standard therapy or without an available standard therapy. ~~**In conjunction with the current ongoing**~~ ***The*** Phase I study of intravenous administration of *C. novyi*-NT spores in patients with treatment-refractory solid tumors [NCT01118819] ***has been closed so that all efforts could be directed to the intratumoral program. T***~~**t**~~he IT treatment protocol will target patients with ***superficial*** tumors measureable, palpable or clearly identifiable under ultrasound or radiographic guidance and amenable to intratumoral injection of *C. novyi*-NT spores.

Rationale: To update the study status of the IV *C. novyi*-NT trial and to reflect the eligibility criteria changes.

1.7 "Management of Adverse Effects Related to Treatment with *C. novyi*-NT "
Second paragraph has been modified.

Changed text:

Preliminary experience from the ongoing canine study also demonstrates that tumor inflammation can be managed by monitoring and ~~**management**~~ ***standard medical support measures.*** Supportive care during the period of time when a localized *C. novyi*-NT infection is being established can result in successful abscess~~**-management,**~~ ***resolution*** as well as subsequent clinical responses. Similarly, application of antibiotics can be delayed in favor of other interventions including, hydration, analgesia, and abscess lancing and drainage.

Rationale: to provide clarity in the AE management.

2.1 "Patient Population"

Changed text:

The study population is patients with solid tumor malignancies that are refractory to standard therapy or without available standard therapy. Men and women ~~**and members**~~ of all races and ethnic groups are eligible for this trial.

Rationale: To simplify the language for patient population.

Section "2.2.1 Inclusion Criteria"
Inclusion criterion #1 has been modified.

Changed text:
Diagnosis of an advanced solid tumor malignancy. There must be a target tumor which is measureable, palpable or clearly identifiable under ultrasound or radiographic guidance and amenable to percutaneous injection of *C. novyi*-NT spores. The targeted lesion must have a longest diameter ≥ 1 cm ***and ≤ 12 cm*** and be measurable as defined by RECIST 1.1 criteria. ***The target lesion must not be located in either the thoracic, abdominal or pelvic cavities or in the brain. There must be no clinical, no functional, and no radiographic evidence of bone involvement at the site of the target lesion.***

Rationale: To achieve greater consistency in the location of lesions injected.

Section "2.2.2 Exclusion Criteria"
Exclusion criterion #7 has been modified.

Changed text:

Treatment with an investigational drug within the past 30 days or 5 half-lives of that drug***, whichever is shorter.***

Rationale: To provide more clarity in Exclusion Criteria 7.

Exclusion criterion #19 has been modified.

Changed text:
Antibiotic allergies ~~**which *that***~~ would preclude treatment for a *C. novyi*-NT infection.~~**, in the event that antibiotics are required.**~~

Rationale: Per the study protocol, all patients will receive antibiotics starting D7.

Section "3. Study Design"
Second paragraph has been modified.

Changed text:
A standard "3+3" dose escalation will be used. The following definitions and rules will be employed:

- Toxicity Criteria will be those listed in the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0, which is available at http://ctep.cancer.gov/reporting/ctc.html
- An adverse event (AE) of Grade 3 toxicity lasting three (3) days or longer or Grade 4 toxicity of any duration that is assessed to be at least possibly related to study agent will be considered a DLT. Signs and symptoms of *C. novyi*-NT infection which are associated with a SOFA score > 5 that require invasive intervention including percutaneous drainage of an abscess or that result in prolonged hospitalization (> 5 days) will also qualify as DLTs.
- ***DLT assessment period is defined as 4 weeks after dosing of the last patient in a cohort.***
- Toxicity will be assessed from administration of study agent up to 8 weeks.
- The following dose escalation rules will apply (See **Table ~~5~~ *2***):
- Additional patients will be enrolled at the MTD to achieve a total of six patients at that dose level if fewer than six patients were treated previously at that dose.
- ~~**Not more than six patients will be treated at any dose level.**~~

- Doses will be increased in successive cohorts until the MTD is reached or until complete germination and tumor regression occurs, making higher doses unnecessary.
- The MTD (or dose at which complete germination and tumor regression occurs) will be the recommended dose for future Phase II *C. novyi*-NT IT administration studies.

Rationale: To clarify that 4 weeks is the required DLT observation period and to align with the 4-week required interval between cohorts (Sec. 3.0). To allow cohort enlargement when indicated by study data.

3.1.1 "Starting Dose Rationale"

Changed text:

The starting dose (Cohort 1) is $1x10^4$ spores administered as a single IT injection. The starting dose is about 700x lower than the Cohort 1 total dose administered in the ~~**current**~~ Phase 1 safety study for intravenous injection of *C. novyi*-NT spores (**NCT01118819**) in which a starting dose of $1x10^5$ spores/kg, equivalent to a total dose of ~$7x10^6$ spores, ~~**is**~~ ***was*** administered ~~**for**~~ ***to*** an average 70 kg patient. The starting IT dose of $1x10^4$ spores is 10,000x lower than the ~~**current dose being used in the ongoing**~~ IT study in companion dogs (**Section 1.5**).

Rationale: To reflect the discontinuation of the IV *C. novyi*-NT study.

3.1.2 Dose Escalation Scheme

Changed text:

Three to six patients will receive IT treatment with *C. novyi*-NT at a dose corresponding to assigned cohort (**Table 3**), starting with Cohort 1. *C. novyi*-NT spores will be administered as an IT injection into the target lesion. Radiographic guidance may be used at the investigator's discretion to aid in targeting an area of necrosis in the target lesion. Patients will be given IV hydration ~~**for 2 hours**~~ after dosing.

Rationale:

To update IV hydration after dosing to be consistent with "Table 4: Schedule of Assessments" for clarity.

3.1.3 Investigational Product and Injection

First and second paragraphs have been modified.

Changed text:

The investigational product, *C. novyi*-NT spores, are packaged in vials and stored in a refrigerator with temperature maintained between 2 – 8 °C. The concentration of spores is [redacted] spores/mL suspended in sterile phosphate buffer saline (PBS). The actual concentration may vary slightly among the GMP lots.

The investigational drug preparation will occur on the day of administration to the patient. The dilution of concentrated spore suspension will be performed at the time of administration in normal sterile saline infusion bags of appropriate size to achieve the required dose based on dose level in each respective cohort. The injectable volume will be 3 mL and will be withdrawn from the saline bag with an appropriate size of syringe (3cc or 5cc) and needle (22-24 gauge). The injection of 3 mL diluted spore suspension may be ***injected with the needle or alternate injection device and the injection can be***

redirected to ***multiple*** distinct sites in the tumor to achieve adequate dispersal of spores throughout the tumor. The injection may be carried out under radiographic guidance.

Rationale:
To update packaging information. To broaden the language to allow the use of alternate injection device for *C. novyi*-NT injection.

Section "3.3 Patient Discontinuation"

First and second paragraphs have been modified.

Changed text:
A discontinuation occurs when an enrolled patient ceases participation in the study, regardless of the circumstances, prior to completion of the protocol. The investigator must determine the primary reason for discontinuation. An attempt will be made by site staff to have all subjects who discontinue ~~**early**~~***prematurely*** from the study complete, at a minimum, the procedures done at the final visit (i.e., the 12 Month visit).

A patient will be discontinued from study if any of the following occur:

- The investigator withdraws the patient from the study in agreement with the Sponsor and Medical Monitor.
- The patient withdraws consent at any time after agent administration. Every effort will be made to determine why any subject withdraws from the study prematurely.
- The patient is lost to follow-up. A genuine effort must be made to determine the reason(s) why a patient fails to return for the necessary visits. If the subject is unreachable by telephone, a registered letter, at the minimum, should be sent requesting him/her to contact the clinic.
- Disease progression in the injected tumor site based on RECIST 1.1 criteria, as assessed by the investigator and not prior to 8 weeks post-dosing. ***(Caution should be taken in assessing progression in the injected tumor prior to 8 weeks since C. novyi-NT germination may cause inflammatory changes resulting in "pseudoprogression".)***
- The patient starts a new anti-cancer therapy or enrolls in another interventional clinical trial.

Rationale: To provide more clarity.

Last 2 paragraphs have been modified.

Changed text:
After discontinuation, further radiographic assessment will not be required and SAEs will no longer be reported, unless they are assessed as related or possibly related to *C. novyi*-NT treatment by the PI. Every effort will be made to collect survival information even after discontinuation, including phone calls on a ~~**bimonthly**~~ ***quarterly*** basis ***for 12 months*** to the patient, patient's relatives or primary oncologist.

All patients who withdraw prematurely prior to ~~84~~ weeks after dosing for reasons other than an AE will be replaced before dose escalation. ***At any time during the study, the Sponsor and Investigators may request that cohorts should be enlarged. Such requests will be discussed with the Sponsor and the Medical Monitor, and should be based on all data existing at that time.***

Rationale: To align with the 4-week DLT observation period and the 4-week required interval between patients (Sec. 3.0). To allow for flexibility in enlarging a cohort based on emerging safety and/or efficacy data.

Section "4.1 Study Calendar"

Pre-treatment (Screening) section has been modified.

Changed text:

Pre-treatment (Screening)	Informed Consent; Medical and Medication History, Physical Exam; Vital signs (BP, HR, respiratory rate, oral body temperature); SpO_2; ECOG; Routine lab work * (including serum pregnancy); Research lab work**; Blood sampling for phosphorus, PT/PTT, uric acid, magnesium, and CRP; CT/MRI Scans; Brain MRI; Echo; ECG; ***weight collection;*** begin AE monitoring

Rationale: To add weight collection to the Screening visit.

Day of admission and treatment (Day 0) section has been modified.

Changed text:

Day of admission and treatment (Day 0)	<u>Pre-dose (day -1 - 0):</u> Medical history, ***P~~p~~***hysical exam; SpO_2; ECOG; ***Blood*** Type (follow unit standards); ECG; Routine lab work* (including serum pregnancy); Cultures ***; Research lab work**; Blood sampling for phosphorus, PT/PTT, uric acid, magnesium, and CRP; ***Pre-dose tumor biopsy (injected lesion) for immunological assessment and tissue banking,*** Vital signs; SOFA; ***weight collection;*** continue AE and concomitant medication monitoring Study agent will be administered as described in the ***Investigational Plan*** section and ***Appendix 1: Study Agent Information***. <u>Post-dose (relative to IT injection):</u> • Continuous SpO_2 monitoring; • Vital signs every 15 minutes (+/- 5 minutes) for 60 minutes, then every 30 minutes (+/- 10 minutes) for 60 minutes, then every 60 minutes (+/- 15 minutes) for 120 minutes. Subsequent vital sign checks will be performed every 4 hours (+/- 1 hour); • Routine lab work at ***60 minutes (+/- 15 minutes) and*** 12 hours post-dose (+/- 2 hours); • Research lab work at ***60 minutes (+/- 15 minutes) and*** 12 hours post-dose (+/- 2 hours); • Blood sampling for phosphorus, PT/PTT, uric acid, magnesium, and CRP at ***60 minutes (+/- 15 minutes) and*** 12 hours post-dose (+/- 2 hours); • Anaerobic blood cultures for *C. novyi*-NT at ***60 minutes (+/- 15 minutes) and*** 12 hours post-dose (+/- 2 hours); • ***Abscess material post-dose for culture, immunological assessment and banking, when performed.*** • SOFA at 8 and 16 hours post-dose (+/- 1 hour)****; • Continue AE and concomitant medication monitoring. • ***IV hydration after dosing (IV fluids and duration are at PI's discretion).***

Rationale:
Pre-dose (day -1 – 0) Section: Blood was added before Type for clarity. To add collection of tumor biopsy on injected lesion for immunological assessment and banking. To add weight collection to the Pre-dose (day -1 - 0).

Post-dose (relative to IT injection) Section: To add the 60 minutes (+/- 15 minutes) collections for routine lab; research lab; blood sampling for phosphorus, PT/PTT, uric acid, magnesium, and CRP; to add IV hydration after dosing and anaerobic blood cultures to the list to be consistent with "Table 4: Schedule of Assessments" for clarity.
To add the collection of abscess material post-dose for culture and banking (when performed) to evaluate immune and inflammatory responses as well as detection of *C. novyi*-NT for correlative studies.

Days 1-7 section has been modified.

Changed text:

Days 1-7	Vital signs every 4 hours (+/- 1 hour) beginning at 24 hours post-dose; Continuous SpO_2 monitoring; SOFA every 8 hours (+/- 1 hour) beginning at 24 hours post-dose****; Physical once daily; ECOG once daily; Routine lab work* once daily beginning at 24 hours post-dose; Blood sampling to measure phosphorus, PT/PTT, uric acid, magnesium, and CRP once daily; Research lab work** once daily; Anaerobic blood cultures for *C. novyi*-NT once daily, ~~**urine cultures (when available) once daily;**~~ CT/MRI Scans (Day 7 only); ***Weight collection (Day 7 only);*** Continue AE and concomitant medication monitoring***; Abscess material for culture, immunological assessment and banking (when performed)***. Patients will be discharged after all procedures/assessments are completed on Day 7.

Rationale: To remove the collection of urine cultures since *C. novyi*-NT is not expected to culture from urine. To add collection of patient weight at Day 7 and collection of abscess material post-dose for culture, immunological assessment and banking (when performed) to evaluate immune and inflammatory responses as well as detection of *C. novyi*-NT for correlative studies.

Outpatient Visits section has been modified.

Changed text:

Outpatient Visits *(Abscess material post-dose for culture, immunological assessment and banking when performed)*

Rationale: To add the collection of abscess material post-dose for culture, immunological assessment and banking (when performed) to evaluate immune and inflammatory responses as well as detection of *C. novyi*-NT for correlative studies.

Day 14, Day 21 section has been modified.

Changed text:

Day 14, Day 21	Physical; Vital signs; ECOG; Routine lab work*; Research lab work**; Blood sampling to measure phosphorus, PT/PTT, uric acid, magnesium, and CRP; ***Weight collection;*** Anaerobic blood cultures for *C. novyi*-NT~~**– NT. Urine cultures when available**~~; Continue AE and concomitant medication monitoring

Rationale: To remove the collection of urine cultures since *C. novyi*-NT is not expected to culture from urine. To add collection of patient weight at Day 14 and Day 21 outpatient visits. To correct *C. novyi*-NT naming error for clarity.

Day 28, Months 2, 4, 8, and 12 section has been modified.

Changed text:

Day 28, Months 2, 4, 8, and 12	Physical; Vital signs; ECOG; Routine lab work*; Research lab work **; Blood sampling to measure phosphorus, PT/PTT, uric acid, magnesium, and CRP; ***Weight collection;*** Anaerobic cultures for *C. novyi*-NT ~~**-NT**~~; CT/MRI Scans; Continue AE and concomitant medication monitoring. ***Tumor biopsy from injected lesion and/or non-injected lesion at month 1 (optional) and month 2 (required) post-dose for immunological assessment, and tissue banking. Biopsy collection and time points may vary depending on tissue availability, local management of tumor inflammation or abscessation, and whether the biopsy can be easily and safely obtained. Biopsy for cultures may also be performed at PI's discretion.***

Rationale: To add collection of patient weight (Day 28 and Months 2, 4, 8, and 12 visits) and collection of tumor biopsies (month 1 and month 2). To correct *C. novyi*-NT naming error for clarity.

Section "4.1 Study Calendar" footnotes

Footnote ** has been modified.

Changed text: **Research lab work includes ***whole blood and*** serum for studies described in the section, ***Correlative Studies***.

Rationale: To expand sample collection types for correlative studies to include whole blood in addition to serum.

Footnote *** has been modified.

Changed text: *** Blood cultures (anaerobic for *C. novyi*-NT and aerobic)~~**; urine when available**~~.

Rationale: To reflect changes in "4.1 Study Calendar" section.

Section "Table 4: Schedule of Assessments"

Table ~~74~~: Schedule of Assessments **Admit Procedure**	**Screen (Day -21 to -1)**	**(Day -1 to 0)**	**Dosing (Day 0)**	**Day 0- Post-dose Time Point (Relative to IT Injection)**												**Days 1 to 7*** (24 h to 192 h)	**Follow-up Day 11, 18, 25** (±1 day)	**Follow-up Day 14, 21** (± 1 day)	**Follow-up Wk 4 to 12 Mon**** (± 2 days)
				15 min	**30 min**	**45 min**	**60 min**	**90 min**	**2 h**	**3 h**	**4 h**	**8 h**	**12 h**	**16 h**	**20 h**				
Eligibility Evaluation																			
Informed Consent	X																		
Inclusion/Exclusion	X																		
Pregnancy Test	X	X																	
Medical History	X	X																	
Brain MRI	X																		
Echocardiogram	X																		
ECG	X	X																	
Safety Evaluation																			
Physical Exam	X	X														X	X	X	X
Vital Signs[e]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X[a]	X	X	X
SpO_2	X	X	X	→	→	→	→	→	→	→	→	→	→	→	→	→			
SOFA Score Asses		X										X		X		X[b]			
ECOG	X	X														X	X	X	X
CBC with differential	X	X					X						X			X[c]		X	X
Serum Chemistry panel[f]	X	X					X						X			X[d]		X	X
Magnesium	X	X					X						X			X		X	X
Phosphorus	X	X					X						X			X		X	X
Uric Acid	X	X					X						X			X		X	X
PT/PTT	X	X					X						X			X		X	X
CRP	X	X					X						X			X		X	X
Weight collection	***X***	***X***														***X (D7)***		***X***	***X***
AE Monitoring	X	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Concomitant Medications	X	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Aerobic blood cultures[1]	X	X																	
Anaerobic blood cultures (for detection of C. novyi-NT)		X					X						X			X		X	X
~~**Urine cultures (if available)**~~		~~**X**~~					~~**X**~~						~~**X**~~			~~**X**~~		~~**X**~~	~~**X**~~
Blood type		X																	
Efficacy Evaluation																			
CT or MRI scans[2]	X															X (**D47**)			X
Serum Banking	X	X					X						X			X		X	X
Whole blood Research[g]		***X***														***X (D1 & D7)***		***X***	***X***
Tumor biopsy[h]		***X***[i]																	***x***[i]
Abscess material[j]				→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Treatment																			
C. novyi-NT			X																
IV hydration			***X***[k]																

ECOG = Eastern Cooperative Oncology Group (Performance Status Scale); SpO_2 = oxygen saturation (by pulse oximetry); SOFA = Sequential Organ Failure Assessment; CBC = complete blood count; PT = prothrombin time; PTT = partial thromboplastin time; CRP = C-reactive protein; ECG = electrocardiogram; AE = adverse event; CT = computed tomography; MRI = magnetic resonance image

* Procedure will be performed either once daily or at the specified time points.

** Visits will be at 28 days and 2, 4, 8, and 12 months (±2 days) after dosing.

a. Vital signs were obtained every 4 hours beginning 24 hours post-dose (i.e., at 24, 28, 32, 36, 40, 44, 48…192 h).

b. SOFA with SpO_2 will be conducted every 8 hours beginning 24 hours post-dose (i.e., at 24, 32, 40, 48…192 h).

c. Blood will be collected for CBC with differential once daily beginning 24 hours post-dose.

d. Blood will be collected for serum chemistry panel once daily beginning 24 hours post-dose.

e. Vital signs will include blood pressure, heart rate, respiratory rate, and oral temperature.

f. In addition to magnesium, phosphorus, uric acid (measured at specified time points), serum chemistry panel will include: glucose, calcium, albumin, total protein, sodium, potassium, lactate dehydrogenase, chloride, blood urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and bilirubin.

g. Whole blood for immunological assessment is collected at Day-1 to 0, D7, D14, D21, week 4, and month 2 (there is no D1 collection); whole blood for banking is collected at Day -1 to 0, D1, D7, D14, D21, week 4 to 12 month follow-up visits, and will be used for C. novyi-NT detection and immunological assessment.

h. Tumor biopsy collections will be done only to those patients who have given consent for tumor biopsy collections and will be used for immunological assessment and tissue banking.

i. Pre-dose tumor biopsy is collected from the injected lesion; Post-dose tumor biopsy (injected and/or non-injected lesions) at month 1 (optional) and month 2 (required) may depend on tissue availability, local management of tumor inflammation or abscessation, and whether the biopsy can be easily and safely obtained. Biopsy for cultures may also be performed at PI's discretion.

j Abscess material samples collected and banked only when performed as part of the management of local tumor inflammation and abscessation.

k. IV hydration after dosing. IV fluids and duration are at PI's discretion.

Rationale: To clarify changes in "4.1 Study Calendar" section and to add whole blood collection for immunological assessment and *C. novyi*-NT detection.

Section "4.2 Safety Evaluation"
First paragraph has been modified.

Changed text*:*
Monitoring for AEs will occur from the time of informed consent until the patient completes his/her participation in the study. The post-dosing observation period will begin with a***n 85*** ~~5~~-day inpatient stay (Days 0 to ***74***). This inpatient stay can be extended and requires that at least 2 days elapse from the resolution of any signs of infection or serious AEs (SAEs; defined in ***Adverse Event Reporting*** below) before discharge.

Rationale: To correct inpatient stay days to be consistent with "4.1 Study Calendar" section for clarity.

Section "4.3 Safety Evaluation Summary"
Summary has been modified.

Changed text:

Days 0 to ***47***	Inpatient observation	
Days ~~5~~***8*** to 28	Twice weekly scheduled visits	Daily phone interviews
Months 2 to 12	Scheduled visits	

Rationale: To correct inpatient stay days and weekly scheduled visits to be consistent with "4.1 Study Calendar" section for clarity.

Section "5. Management of Study Agent-related Toxicity"
Last paragraph has been modified.

Changed text:
Need for surgical drainage will be assessed on a case-by-case basis, and surgical consultation will be requested as the case dictates. ***When surgical drainage of the tumor is conducted a material sample will be collected for culture and banking.***

Rationale: To reflect changes in "4.1 Study Calendar" section for clarity.

Section 6.5 Serious Adverse Event Reporting
Second paragraph has been modified.

Changed text:
Serious AEs must be reported ***as soon as possible after the PI or investigator has become aware of its occurrence by submitting a completed SAE form*** to ~~the Medical Monitor~~ ***PAREXEL*** [redacted] ***by fax at*** [redacted] ***or via email at*** [redacted] ~~by telephone as soon as possible after the PI or investigator has become aware of its occurrence.~~

~~**Medical Monitor**~~
PPD
PPD
~~PAREXEL International~~

PPD
PPD
PPD
~~Fax:~~ PPD
~~Email:~~ PPD

In the event that the site is unable to complete the SAE form to report the event within 24 hours of their becoming aware of the event, the investigators must report the SAE over the telephone via the SAE answering service at CCI ***, and then provide the completed SAE form via fax or email.***

~~The PI/investigator must complete an SAE form and submit it to PAREXEL – North American Medical Services within 24 hours of becoming aware of the event.~~

~~Completed SAE forms should be faxed to~~ CCI ~~. Alternatively, investigators may report SAEs over the telephone via the SAE hotline~~ CCI ~~; they will be required to provide the completed SAE forms within the next 24 hours.~~

Rationale: To reflect update contact and procedure changes in SAE reporting.

Section 7. "Efficacy Evaluation"

Changed text:

Response and progression will be evaluated using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee (version 1.1: http://www.eortc.be/recist/).

Objective responses will be measured by serial CT or MRI scans of the injected tumor and sites of metastatic involvement. Overall ~~**tumor**~~ response, based on CT/MRI scan results, will be based on observation of measurable and non-measurable disease as compared to baseline and nadir in target and non-target tumors per RECIST 1.1. ***CT or MRI scans may be submitted to a third party vendor for review.***

Rationale: To provide consistency with the study objective 2.

Section "8. Correlative Studies"
First paragraph has been modified.

Changed text:
~~**From the collection of serial blood**~~ ***Correlative*** samples~~**, serum**~~ will be ~~**isolated**~~ ***collected*** and stored ~~(~~for up to 5 years~~)~~. ***Whole blood samples will be collected for immunological assessments and C. novyi-NT detection. When performed, post-injection site abscess material samples will be collected and stored (for up to 5 years). Archived tissue may be used for correlative studies provided that informed consent from the subject is obtained, or when not possible, a waiver of consent is granted by the IRB. Comprehensive information on sample acquisition, handling and storage are to be found in the study manual. Sample tube labels should include the patient identification number/protocol code, sample number and visit number and will be detailed in the study laboratory manual. Refer to study manual for samples storage conditions at the study center until shipment under appropriate conditions to the third party laboratory vendor and/or sample storage location.*** Samples will be used to evaluate the following ***parameters:***

Rationale: To reflect changes in "4.1 Study Calendar" section and provide general sample collection information for clarity.

Section "8.1 Measurement of the anti-*C. novyi*-NT immune and inflammatory responses"
First paragraph has been modified.

Changed text*:*
The immune and systemic inflammatory responses during *C. novyi*-NT treatment will be evaluated by serial measurement of anti-*C. novyi*-NT antibodies, CRP, ~~and~~ selected cytokines***, and analysis of selected immune cell populations in blood***. C-reactive protein and certain cytokines (IL-6, IL-8, TNF-alpha, etc.) are markers of systemic inflammation. ~~**These will be measured using commercially available kits.**~~ Patient ***whole blood and*** serum ***samples*** will be obtained as indicated on the study calendar ***and, when performed, pre-dose and post-dose tumor samples and abscess material will be collected.*** ~~**, and will be screened at the end of the study in batch. Whole blood (6 mL) will be collected in a serum separator tube (SST) at the designated time points and processed using standard procedures for serum separation. Serum will be divided into 1 mL aliquots and stored at -80°C.**~~

Rationale: To reflect changes in "4.1 Study Calendar" section and to remove detailed sample collection information. The general sample collection information was moved to the beginning of section 8.

Section "8.2 *C. novyi*-NT detection in circulation"
Section title and first paragraph has been modified.

Changed text*:*
8.2 *C. novyi*-NT detection in circulation *and/or abscess material/post-dose tumor sample*

Scheduled ~~**patient**~~ ***whole blood*** sampling will take place as indicated in the study calendar (section 4.1) and Table 4. Anaerobic blood cultures ***and/or abscess material/post-dose tumor sample (when performed)*** will be assessed to determine the presence ~~**or absence**~~ (~~**clearance**~~from circulation) of *C. novyi*-NT.

Rationale: To reflect changes in "4.1 Study Calendar" and study objectives sections.

Section 10.4 Good Clinical Practice
Changed text*:*

The clinical study also will be carried out in keeping with national and local legal requirements (in accordance with United States Code of Federal Regulations [21 CFR 312.50 and ***21 CFR*** 56]) and abiding by the ~~**principles**~~ ***guidelines*** of the ICH guidelines on GCP and ***the moral, ethical and scientific principles governing clinical research as set out in*** the Declaration of Helsinki (Version 2008).

Rationale: To provide more clarity in GCP.

Section "11.1 Patient Information and Informed Consent"
New paragraph at the end of the section was added.

Added text*:*
Separate consent will be requested to obtain/retain blood, serum and abscess material/biopsy

samples (when available). Samples will be stored for 5 years for future analysis, which may include genomic evaluation of the tumor tissue, as warranted by our rapidly-advancing understanding in this field. Separate consent will also be requested to obtain, when possible, photographs of the tumor pre- and at various time points post-dose.

Rationale: To update patient consent to reflect the expanded sample collection for future analysis.

Appendix 1: Study Agent Information

Changed text:

Packaging/Labeling

~~**The Johns Hopkins Cell Processing and Gene Therapy Facility (CPGT)**~~ ***Omnia Biologics, Inc.*** is responsible for the manufacturing and for the formulation of *C. novyi*-NT spores. The *C. novyi*-NT spores are packaged in ~~**threaded cryovials**~~ ***glass vials*** at a concentration of approximately CCI spores/mL suspended in sterile PBS and will be labeled with the following information: identity of test article, number of particles contained and volume, directions for use, storage requirements, caution statement, lot number.

Storage and Security Conditions

~~**Cryovials**~~***Vials*** of *C. novyi*-NT spores will be stored at 2-8^{o}C controlled temperature environment under constant temperature monitoring with access limited to authorized study personnel.

Study Agent Preparation

The preparation of the injectable drug suspension will take place on the day of IT administration. The ***vial*~~**cryovial**~~(s) containing *C. novyi*-NT spores will be ~~**unsealed and**~~ diluted using the appropriate volume of sterile 0.9% sodium chloride (NaCl). *C. novyi*-NT spore preparation and dilution will take place in a designated biological safety cabinet (BSC) and may be carried out at room temperature. The vial should be vortexed to ensure an even suspension of spores before the appropriate volume of aliquot is drawn for dilution. The appropriate volume of spores will be drawn from the vial and added into an appropriate size of sterile saline infusion bag for dilution. The resulting saline bag will be inverted multiple times to ensure that the spores are mixed completely with the saline and there are no visible particulates. 3 mL of the diluted spore suspension will be withdrawn from the saline bag with an appropriate size of syringe (3 mL or 5 mL) for injection. The proper volume of the concentrated spores and the proper size of the saline bag for dilution will be determined by the dose level for respective cohorts, and the exact information will be included in the Investigational Drug Manual.

Study Agent Administration

The study agent will be administered starting with Cohort 1 at a dose of 1 x 10^4 spores followed by dose escalation as described in the *Study Design (***Section 3***)*. The patient will be given IV hydration ~~**for 2 hours**~~ after dosing. ***IV fluids and duration are at PI's discretion.***

Study Agent Disposal

In the event of a spill of the study agent, a clean-up protocol exists (***Appendix 2: Study Agent Spill Procedure***)***.*** This protocol and the supplies necessary will be on hand at the time of study agent administration. Otherwise, dosing administration supplies will be collected and sealed in a biohazard bag and destroyed.

The ***vial*~~**cryovial**~~(s) and dilution bag will be destroyed per the institution's standard for biohazard waste.

Rationale: To update manufacturing location and packaging information. To update IV hydration after dosing to be consistent with "Table 4: Schedule of Assessments" for clarity.