Phase I clinical study of intratumoral injection of oncolytic *Clostridium novyi*-NT spores in patients with advanced cancers

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BACKGROUND

Intratumoral injection of *Clostridium novyi*-NT (*C. novyi*-NT), an attenuated strain of Clostridium, induced a microscopically precise, tumor-localized response in a rat orthotopic brain tumor model, in companion dogs bearing spontaneous solid tumors, and in the first patient treated on a Phase 1 human clinical trial (Roberts et al, Sci Transl Med. 2014) [1]. *C. novyi*-NT spores germinate in hypoxic tumor environment and lyse malignant cells by secreting lipases, proteases, other hydrolytic enzymes, and recruiting inflammatory cells to tumors eliciting anti-tumor immune responses in animals[2]. Furthermore, intratumoral injection can plausibly induce an immune mediated abscopal effect in non-injected tumor sites.

METHODS

Study Design

Objectives

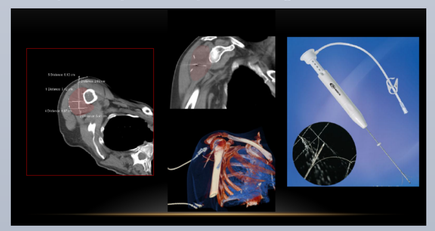
- To determine the maximum tolerated dose (MTD), and dose-limiting toxicities (DLT) of a single intratumoral injection of *C. novyi*-NT using the standard 3+3 dose escalation schedule
- To document preliminary anti-tumor activity of both the injected tumor and an overall response
- To study the presence of circulating *C. novyi*-NT spores
- 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

Major eligibility criteria

- Adult patients with advanced solid tumor malignancy
- Targeted tumor has a longest diameter ≥ 1 cm and ≤ 12 cm and is amenable to percutaneous injection of *C. novyi*-NT spores.
- The target tumor must not be located in either the thoracic, abdominal, pelvic cavities or in the brain.
- ECOG 0-2
- ANC ≥ 1,000/uL
- Hemoglobin ≥ 9 g/dL
- Platelets ≥ 100,000/uL
- Total bilirubin ≤ 1.5 x upper limit of normal (ULN)
- ALT/AST ≤ 2.5 x ULN
- INR ≥ 1.3
- No primary brain malignancies or brain metastases
- No active infection or treatment with antibiotics

Intratumoral Injection

C. *novyi-NT* spores are injected with a needle or alternate injection device, which may be carried out under radiographic guidance. The injection can be redirected to multiple distinct sites in the tumor to achieve adequate dispersal of spores throughout the tumor.



RESULTS

Dose Escalation

Dose Level	Dose of Intratumoral *C. Novyi*-NT Spores	Number of patients Enrolled	Dose-limiting toxicities (DLT)
1	1 x 10^4	3	None
2	3 x 10^4	3	None
3	10 x 10^4	4	None
4	30 x 10^4	6	1 (sepsis)
5	100 x 10^4	3 Planned cohort expansion to 6 patients to determine MTD	None
6	300 x 10^4	2	2 (sepsis, gas gangrene)

Patients Characteristics (n=21)

Median age – years (range)	55 (29-75)
Male/Female	11/10

The Type, Location, Size, and Response of Injected Tumors

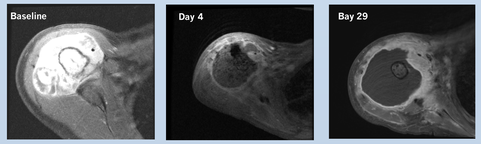
Patient	Cohort	Tumor Type	Location	Size (cm)
011-001	1	Leiomyosarcoma	Peri-humerus Soft Tissue (right)	7.6*
011-002	1	Chondrosarcoma	SQ Chest Wall (Left)	2.7
011-003	1	Leiomyosarcoma	SQ Abdominal Wall (Left)	2.6
011-004	2	Mullerian Carcinoma	SQ left flank	10.5
008-006	2	Angiosarcoma	SQ neck lymph node	2.8
011-007	2	Papillary thyroid Carcinoma	SQ Scapular Lesion	3
006-010	3	Breast cancer (Ductal Carcinoma)	Skin Lesion	3
008-012	3	Serous Ovarian Cystadenocarcinoma	Axillary Mass	5
008-013	3	Oropharyngeal Cancer (HPV-related Squamous Cell Carcinoma)	Pre-auricular Lymph Node	2
011-014	3	Breast Cancer (Triple Negative adenocarcinoma)	Left Chest Wall Superficial Lesion	3
011-016	4	leiomyosarcoma	Right Abdominal Wall	5
008-018	4	Metastatic Osteosarcoma	Right Leg Amputation Site	8*
011-019	4	Liposarcoma	Right Groin	3*
011-021	4	Endometrial Carcinoma	Abdominal Wall	3
003-022	4	Sarcoma	Left Lateral Abdominal Wall	3.7
011-023	4	Adenocarcinoma	Axilla	10
008-026	5	Chordoma	Chest Wall (Left Posterior)	2.8
011-027	5	Spindle Cell Sarcoma	Right Arm	4.5*
011-028	5	NRAS-mutated Melanoma	Abdominal Wall	2.5
008-029	6	Leiomyosarcoma	Right Anterior Hip	8.5*
008-030	6	Myxofibrosarcoma	Bicep	10*

Green Fill: C. *novyi-NT* germination/tumor destruction * SAE

Best Response

- Anti-cancer activity was observed in 10 out of 21 (48%) patients in whom *C. novyi-NT* germination resulted in extensive, in some cases, complete central necrosis of the injected tumor.
- Two patients had 22-24% shrinkage in the injected tumor at Month 1-2 based on RECIST assessment. One patient has 14% shrinkage in the injected tumor at Month 4.
- Out of the 10 patients who were evaluated at Month 2, 8 patients had an overall response of stable disease; 2 patients had progressive disease. One patient had stable disease at Month 4.
- Three out of 21 patients had no visible signs of C. *novyi-NT* germination but experienced dramatic clinical improvement at Month 1 (Patient 008-013) or slowed tumor growth at Month 2 as compared with tumor growth before C. *novyi-NT* injection (Patient 011-028, 008-026).

Patient 011-001: 53-year-old female with a 7.6 cm leiomyosarcoma in the right shoulder with adjacent humerus involvement. Patient was treated at Dose Level 1 (1x10^4 spores).[1]

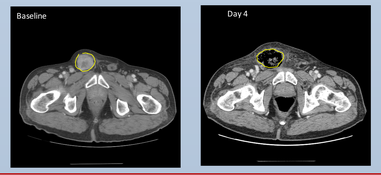


Baseline: contrast enhancing mass involving soft tissue and possibly adjacent bone. Lesion measures about 7.2cm AP x 7.4cm transverse in the axial dimensions.

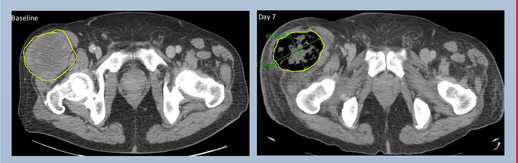
Day 4: markedly diminished contrast enhancement within the tumor mass of soft tissue and possibly adjacent bone component

Day 29: non-enhancing tumor mass becomes more homogeneous consistent with ongoing necrosis

Patient 011-019: 75-year-ol male with a 3 cm liposarcoma in the right inguinal node. Patient was treated at Dose Level 4 (30x10^4 spores).

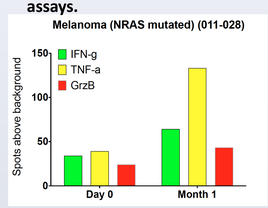


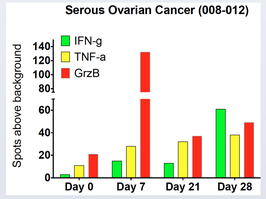
Patient 008-029: 65-year-old male with a 8.5 x7 cm metastatic right anterior hip leiomyosarcoma. Patient was treated at Dose Level 6 (300x10^4 spores)



Tumor-specific T-cell Response

To assess tumor-specific T-cell responses in patients treated with C. *novyi-NT* injection, circulating CD4 and CD8 cells were isolated from patient's blood and stimulated with patient's own dendritic cells which were pulsed with tumor antigens. Release of T-cell cytokines and effector molecules such as IFN-γ, granzyme-B, and TNF-α were quantified by Enzyme-linked Immune Absorbent Spot (ELISPOT) assays.





Toxicity Data Summary

Most common adverse events (AEs) are hyperthermia/fever and tumor inflammation. Fever is expected due to the nature of the therapy and has been observed in half of treated patients. Tumor inflammation and abscess events are generally considered desired mechanisms of C. *novyi-NT* treatment.

Related Grade 3 and Above AEs and Serious Adverse Events (SAEs)

Patient ID	Adverse Event	Severity	Cohort Dose
011-001	Respiratory Insufficiency	Grade 3	Cohort 1
011-001	Pathologic Fracture of Right Humerus	Grade 3	Cohort 1
008-018	Sepsis	Grade 4	Cohort 4
011-019	Genital Swelling	Grade 2	Cohort 4
011-023	Pain	Grade 3	Cohort 4
011-023	Swelling	Grade 3	Cohort 4
011-023	Possible Skin Infection	Grade 3	Cohort 4
011-027	Abscess	Grade 3	Cohort 5
008-029	Sepsis	Grade 4	Cohort 6
008-029	Soft Tissue Infection	Grade 3	Cohort 6
008-030	Gas Gangrene Right Upper Extremity	Grade 4	Cohort 6

Green Fill: SAEs

CONCLUSION

- A single dose of intratumoral injection of *Clostridium novyi*-NT is feasible and has led to significant destruction of injected tumor masses.
- Stabilization/slow down in tumor growth in non-injected masses has been observed in two patients.
- The tolerable dose has been exceeded, and expansion of cohort 5 is planned to define MTD and RP2D.
- A Phase I study of a PD1 antibody with *C. novyi*-NT has been designed.

1 Roberts, N, Zhang, L, et al. (2014). Intratumoral injection of *Clostridium novyi*-NT spores induces antitumor responses. Sci Transl Med. 6(249):249ra111
2 Agrawal N, Bettegowda C, et al. (2004) Bacteriolytic therapy can generate a potent immune response against experimental tumors. Proc Natl Acad Sci U S A 101:15172-7