

PHARMACOKINETICS OF INTRAVENOUSLY ADMINISTERED SENECA VALLEY VIRUS (NTX-010) IN A PHASE I TRIAL OF PATIENTS WITH SOLID TUMORS OF NEUROENDOCRINE DIFFERENTIATION

Kevin Burroughs², Seshidhar Reddy², Charles M. Rudin¹, John R. Neefe², Lawrence Garbo³, Joe Stephenson³, David Loesch³, Daniel Von Hoff³, David Smith³, Donald Richards³, Paul Conkling³, Carlos Alemany³, Barbara Coleman¹, and Paul Hallenbeck²



¹Johns Hopkins University, Baltimore, MD USA, ²Neotropix, Inc., Malvern, PA USA, ³US Oncology, Houston, TX USA

ABSTRACT

Background Seneca Valley Virus (SVV-001/NTX-010) is a newly discovered, replication-competent picornavirus with natural oncolytic selectivity towards human tumor cells of neuroendocrine (NE) differentiation, including small cell lung cancer (SCLC), carcinoid and pediatric solid tumors.

Methods Neotropix initiated a Phase 1 dose-escalation study in May, 2006, for patients with solid tumors with NE features. Virus was administered as a single intravenous infusion over one hour. Five dose levels were planned at log increments from 10⁷ to 10¹¹ vector particles/kg. The objective of the trial was to determine a recommended dose for phase 2. As part of this trial, viral kinetics in serum and in four other compartments—urine, stool, sputum and nasal swab—and neutralizing antibodies in serum were monitored to assess NTX-010 replication and clearance and host immunity to the virus. Viral load in serum was monitored using a cell-based infectivity assay and a quantitative real-time RT-PCR assay. Anti-viral host immunity was monitored in serum using a cell-based virus neutralization assay.

Results Eighteen patients with small cell carcinoma, carcinoid or other NE tumors expressing NE markers were enrolled. An interim safety analysis was conducted in November 2007, and these patients form the population for the current report. Varying patterns of serum kinetics of NTX-010 were observed. In all but two patients, NTX-010 was detected in serum with kinetics consistent with viral replication in these patients. All patients followed for at least 30 days cleared virus from all compartments and developed a detectable serum neutralization response to NTX-010 with an observed dose-dependent trend in the rate and amplitude of the response.

Conclusion Viral kinetics are consistent with NTX-010 replication in most patients with tumors with NE features and all patients followed for at least 30 days cleared virus from all compartments. All patients developed detectable neutralizing antibodies.

BACKGROUND

Oncolytic viruses offer the potential to kill cancer cells, without harming normal cells, based on the molecular specificity of viral host cell tropism which allows for tumor-selective replication and cell lysis. While proof of concept and some promising results have been obtained treating localized disease via intratumoral injection, systemic treatment for disseminated disease has not been highly successful. This is due in part to the fact that most oncolytic virus platforms are based on human pathogens. As a result toxicity and pre-existing antibodies have limited the use of oncolytic viruses as systemic therapies.

NTX-010 is a recently discovered picornavirus that is believed to be a non-pathogenic virus with natural tropism to swine. NTX-010 offers the advantage of not being inhibited by any component of human blood, and is therefore being developed as a systemically deliverable oncolytic virus for human cancers. *In vitro* characterization of NTX-010 cytotoxicity on human cells indicated that NTX-010 has no effect on any normal adult cell tested, but potently kills human tumor lines from carcinoid, SCLC, pediatric cancers (neuroblastoma, retinoblastoma, medulloblastoma, Ewing's sarcoma, rhabdoid tumors and Wilms' tumor). These tumor types typically express markers of NE differentiation, suggesting that the molecular determinant(s) of tropism may be part of the genetic program of the transformed NE phenotype. Testing in preclinical models demonstrated the anti-tumor activity of NTX-010 in a wide array of NE tumors.

METHODS

Objectives

- Primary**
- To evaluate safety and tolerability and define the recommended phase II dose for i.v. infusion of NTX-010 in patients with advanced solid tumors with neuroendocrine features.
- Secondary**
- To characterize viral load, distribution and elimination in serum, sputum, stool, urine, and nasal swab.
 - To evaluate serum neutralization response to NTX-010.
 - To document intratumoral viral replication of NTX-010 *in vivo*.
 - To obtain preliminary information of anti-tumor activity of NTX-010 in this patient population.

Patient population

Two primary cohorts of patients have been studied to date: a dose-escalation cohort in patients with any NE cancer with estimated survival ≥6 months, and an expansion cohort of advanced SCLC (survival ≥3 months) treated at 10⁷ vp/kg.

Dose escalation cohorts

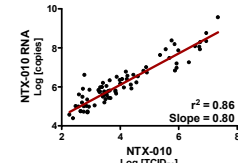
| Cohort | NTX-010 (vp/kg) | N (to date, 18 total) |
|--------|--------------------------------|-----------------------|
| 1 | 10 ⁷ × 1 dose only | 2 |
| 2 | 10 ⁸ × 1 dose only | 3 |
| 3 | 10 ⁹ × 1 dose only | 4 |
| 4 | 10 ¹⁰ × 1 dose only | 3 |
| 5 | 10 ¹¹ × 1 dose only | 0 |

SCLC expansion 10⁷ × 1 dose only 6
Eight of the 12 patients in the NE dose-escalation cohort had carcinoid.

RESULTS

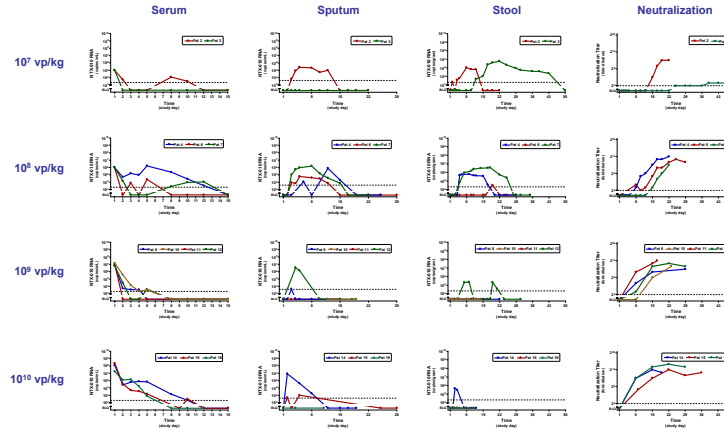
Comparison of Infectivity and qRT-PCR Assays

NTX-010 was measured in patient samples using both a functional, cell-based infectivity (TCID₅₀) and qRT-PCR assays, which detects viral genomic RNA. Assay values from specimens for which both the assays returned results above the lower limit of quantification were plotted and subjected to linear regression. Due to the difference in sensitivity between the two assays, numerous samples positive by qRT-PCR were below the limit of quantification for infectious virus. A stool sample was the only specimen for which infectious virus was detected and no qRT-PCR signal was observed.

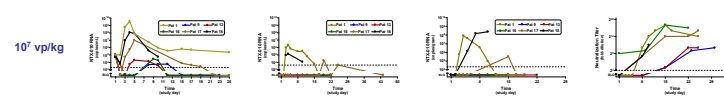


Viral Load in Patients

6-Month Arm



3-Month Arm



NTX-010 viral load in serum, sputum, stool, urine and nasal swabs from patients was determined by qRT-PCR. Anti-viral neutralization titer in serum was determined using a cell-based infectivity assay. Assay values are plotted versus time for each dose cohort in the two trial arms. NTX-010 was administered on Day 1. Serum viral titers on Day 1 were determined from blood collected during the final 5 minutes of the one-hour infusion. Urine and nasal swab samples were only sporadically positive for NTX-010.

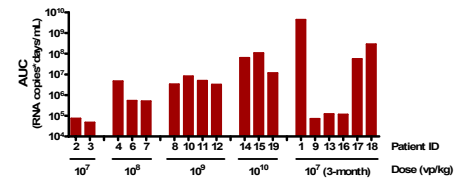
Viral load monitoring

- Strong correlation existed between results from infectious virus and qRT-PCR assays
- qRT-PCR provides a more sensitive, reproducible and efficient method of NTX-010 detection in samples monitored

Viral replication

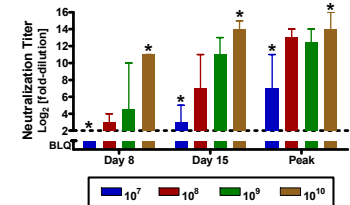
- Viremia was detected in post-dose serum samples from 16 of 18 patients
- Virus was not detected in any compartment from Patient 11, suggesting lack of viral tropism in some patients
 - 6-month arm
 - Kinetic patterns observed in serum are consistent with and suggestive of replication in 10 of 12 patients
 - Increase in total exposure (AUC) with dose approximates that of robust replication in SCC patients
 - 3-month arm
 - Peak serum levels in Patients 1, 17 and 18 provide unequivocal evidence of viral replication
 - The specificity of robust replication for patients with SCC is suggestive of tumor-selectivity of NTX-010

Total Exposure to NTX-010



Area under the curve (AUC) for viral load in serum was determined using GraphPad Prism 4.2 software to assess total exposure to NTX-010 for patients in both the 6-month and 3-month trial arms. Baseline was set at the lower limit of quantification in serum (2.14x10³ copies/mL).

Neutralization Response is Dose-dependent



Neutralization titers for patients in the 6-month trial arm are plotted for Days 8 and 15 and peak titer while on study. A trend for a more rapid and robust neutralization response was observed with increasing dose. Significant differences between mean titers for 10⁷ and 10¹⁰ vp/kg cohorts were observed on Day 8, Day 15, and peak (*P<0.05, one-way ANOVA and Tukey's multiple comparison test on log₂-transformed values).

SUMMARY and DISCUSSION

Viral shedding

- Infectious NTX-010 was shed in sputum and stool
 - A trend towards decreased shedding with increasing dose was observed (6-month arm)
 - Shedding in stool and sputum suggests some replication occurs in gastrointestinal tract
- All patients followed for at least 30 days cleared virus from all compartments

Neutralization response to NTX-010

- All patients developed a measurable neutralization response to NTX-010
- Neutralization response was dose-dependent (6-month arm)
- Patient 16 was the only patient to date with pre-existing neutralization titer
 - Specific antibodies to NTX-010 were confirmed by ELISA
 - Pre-existing antibodies are suggestive of previous exposure to NTX-010 or similar virus without overT toxicity