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Pediatric Preclinical Testing Program (PPTP) evaluation of the oncolytic picornavirus, NTX-010 (SVV-001)

Christopher Morton[1], Peter J. Houghton[1], John M. Maris[2], Richard Gorlick[3], E. Anders Kolb[4], Min H. Kang[5], C. Patrick Reynolds[5], Malcolm A. Smith[6].
[1]St. Jude Children's Research Hospital, [2]Children's Hospital of Philadelphia, [3]Albert Einstein College of Medicine, [4]A.I. duPont Hospital for Children, [5]Texas Tech University Health Sciences Center, [6]CTEP/NCI.

Abstract

Background: NTX-010 is a novel oncolytic picornavirus with antitumor activity against human cancers expressing neuroendocrine markers. The activity of NTX-010 was evaluated against the in vitro and in vivo panels of the Pediatric Preclinical Testing Program (PPTP).

Methods: The PPTP includes a molecularly characterized in vitro panel of cell lines (n=27) and in vivo panel of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood acute lymphoblastic leukemia (ALL). NTX-010 was tested against the PPTP in vitro panel at concentrations ranging from 10^{4} virus particles per cell to 10^{-4} virus particles per cell and was tested against the PPTP in vivo panels at a dose of $3X10^{12}$ virus particles per kg administered as a single dose via intravenous injection. Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event (4-fold increase in tumor volume) measure based on the median Event Free Survival (EFS) of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).

Results: NTX-010 was variably active against lines in the in vitro panel with activity focused in the Ewing, neuroblastoma and rhabdomyosarcoma histologies, while no activity was observed against leukemia and lymphoma lines. NTX-010 achieved objective responses in 12 of 35 xenografts tested (34%) with objective responses in the Wilms, rhabdoid, glioblastoma, neuroblastoma and alveolar rhabdomyosarcoma panels. Activity was greatest for the neuroblastoma and alveolar rhabdomyosarcoma panels. In the neuroblastoma panel, there was 1 partial response and 3 maintained complete responses (MCRs) among 5 xenografts tested. Each of the 4 alveolar rhabdomyosarcoma xenografts tested achieved MCRs. NTX-010 was not evaluated against the ALL in vivo panel.

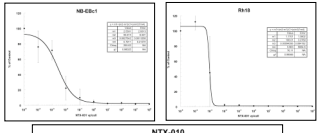
Conclusions: NTX-010 demonstrated activity against the PPTP's in vitro and in vivo solid tumor panels, with activity concentrated in models expressing neuroendocrine markers (e.g., NCAM1). Particularly notable was the high level of in vivo activity observed for neuroblastoma and alveolar rhabdomyosarcoma panels. Further studies characterizing molecular predictors of response and the activity of combinations of NTX-010 with other anticancer agents are anticipated. (Supported by NCI NO1CM42216)

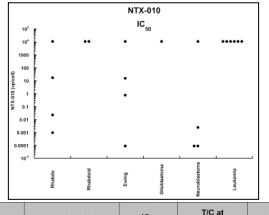
PPTP *In Vitro* Testing Methods

Methods: *In vitro* testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viable (using fluorescein diacetate [FDA]) cell numbers in tissue culture multiwell plates (Keshelava, et al. Methods Mol.Med., *110*: 139-153, 2005). Testing was for 96 hours at concentrations from 10^{-4} virus particles per cell to 10^{4} virus particles per cell, with replicates of 6 per data point. Data were analyzed using Kaleidagraph (Synergy), fitting a non-linear regression, sigmoidal dose-response model to the response, relative fluorescence values vs. the concentration. The PPTP in vitro panel contains cell lines for neuroblastoma (4), Ewing sarcoma (4), rhabdomyosarcoma (4), ALL (5), NHL (2), and others.

NTX-010 *In Vitro* Activity

- Three of 4 neuroblastoma, 2 of 4 rhabdomyosarcoma, and 1 of 4 Ewing sarcoma cell lines had IC_{50} value less than 1 virus particle per cell
- At the highest concentration tested ($1x10^{4}$ vp/cell), 9 of the 23 cell lines showed > 90% inhibition compared to control cells.
- NTX-010 demonstrated no cytotoxic effect against cell lines of lymphoid or myeloid origin.





Cell Line	Histology	IC_{50} (vp/cell)	T/C at 1 vp/cell (% of control)	T/C at 1E+04 vp/cell (%of control)
RD	Rhabdomyosarcoma	>1.0E+04	99.43	97.58
Rh41	Rhabdomyosarcoma	7.29E-02	1.74	0.47
Rh18	Rhabdomyosarcoma	9.66E-04	1.13	0.54
Rh30	Rhabdomyosarcoma	1.75E+01	89.56	8.08
BT-12	Rhabdoid	>1.0E+04	95.63	100.00
CHLA-266	Rhabdoid	>1.0E+04	100.00	55.79
TC-71	Ewing sarcoma	1.57E+01	95.17	0.00
CHLA-9	Ewing sarcoma	<1.0E-04	0.01	0.00
CHLA-10	Ewing sarcoma	7.73E-01	56.20	0.02
CHLA-258	Ewing sarcoma	>1.0E+04	100.00	59.15
GBM2	Glioblastoma	>1.0E+04	100.00	33.84
NB-1643	Neuroblastoma	<1.0E-04	0.02	0.01
NB-EBc1	Neuroblastoma	2.46E-03	4.89	1.50
CHLA-90	Neuroblastoma	>1.0E+04	98.92	61.26
CHLA-136	Neuroblastoma	<1.0E-04	1.31	0.44
NALM-6	ALL	>1.0E+04	81.66	83.20
COG-LL-317	ALL	>1.0E+04	100.00	100.00
RS4;11	ALL	>1.0E+04	91.71	83.37
MOLT-4	ALL	>1.0E+04	100.00	96.59
CCRF-CEM	ALL	>1.0E+04	100.00	85.73
Kasumi-1	AML	>1.0E+04	100.00	82.78
Karpas-299	ALCL	>1.0E+04	93.42	83.51
Ramos-RA1	NHL	>1.0E+04	100.00	89.11

Methods for PPTP *In Vivo* Testing

Stage 1 testing involves testing an agent across the entire PPTP panel of childhood cancer xenograft lines at its MTD or at a dose selected based on PK/PD studies using adult preclinical models.

Solid tumor testing: For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2–0.5 cm^3. Two perpendicular tumor diameters were measured at once weekly intervals with digital vernier calipers. Assuming tumors to be spherical, volumes were calculated from the formula $(\pi/6)\times d3$, where d represents the mean diameter.

Acute lymphoblastic leukemia testing: For each xenograft line, 8 mice were inoculated with 3-5 x 10^{6} mononuclear cells purified from the spleens of secondary recipient mice. Engraftment was monitored weekly by flow cytometry, and treatment was initiated when the proportion of human CD45+ cells in the peripheral blood reached 1%. The proportion of human CD45+ cells in the peripheral blood was monitored weekly throughout the course of treatment.

Drug: All experiments reported were approved by the institutional biological safety committee of each institution and were performed with adherence to each institute's guidelines. NTX-010 was provided by Neotropix (Malvern, PA). NTX-010 was diluted in Dulbecco's PBS, and administered intravenously as a single dose of $3X10^{12}$ virus particles/kg.

Solid Tumor Response Criteria:

Response	Definition	Score
PD1 (Progressive Disease 1)	>25% ↑in tumor volume, TGD value ≤1.5	0
PD2 (Progressive Disease 2)	>25% ↑in tumor volume, TGD value >1.5	2
SD (Stable Disease)	<25% ↑in tumor volume, <50% regression	4
PR (Partial Response)	≥50% regression, but no CR	6
CR (Complete Response)	<0.1 cm3 tumor volume	8
MCR (Maintained CR)	<0.1 cm3 tumor volume at the end of study	10

Leukemia Response Criteria:

Response	Definition	Score
PD1 (Progressive Disease 1)	No PR & TGD value of ≤1.5 & events at EOS	0
PD2 (Progressive Disease 2)	No PR & TGD value >1.5 & events at EOS	2
SD (Stable Disease)	No PR and no events at EOS	4
PR (Partial Response)	CD45% <1% for only 1 week	6
CR (Complete Response)	CD45% <1% for 2 consecutive weeks	8
MCR (Maintained CR)	CD45% <1% for last 3 weeks of study	10

Median Group Response: Each individual mouse in the treatment group was assigned a response score (see Tables above) and a median score for the treatment group was calculated and then each treatment group was assigned an overall response according to the table below.

If Median Score (MS) from (1):	Overall Group Response
0 ≤ MS ≤1	PD1
1 < MS ≤3	PD2
3 < MS ≤5	SD
5 < MS ≤7	PR
7 < MS ≤9	CR
9 < MS	MCR

Statistical Methods: Event-free survival (EFS) distributions of each treatment group were compared to the EFS distribution of the respective control group using the exact log rank test. P-values were 2-sided & were not adjusted for multiple comparisons given the exploratory nature of this study. P-values < 0.05 were considered to be significant.

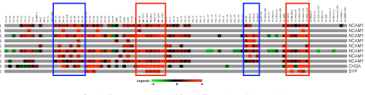
NTX-010 *In Vivo* Activity

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	Overall Group Response
BT-29	Rhabdoid	<0.001	> 2.4	3.7	0.58	0.002	PD2
KT-14	Rhabdoid	<0.001	> 2.1	0	0.05	<0.001	MCR
KT-12	Rhabdoid	0.012	1.3	>4	0.79	0.136	PD1
KT-10	Wilms	<0.001	1.7	>4	0.51	<0.001	PD2
KT-11	Wilms	0.002	1.2	>4	0.66	0.005	PD1
KT-13	Wilms	<0.001	> 2.0	0.6	0.14	<0.001	PR
SK-NEP-1	Ewing	<0.001	2.5	>4	0.4	<0.001	PD2
EW5	Ewing	0.013	1.4	>4	0.78	0.075	PD1
EW8	Ewing	0.248	1.3	>4	0.88	0.604	PD1
TC-71	Ewing	0.944	1.1	>4	0.59	0.035	PD1
CHLA258	Ewing	0.104	0.9	>4	1.25	0.19	PD1
Rh10	ALV RMS	<0.001	> 2.0	0	0.05	<0.001	CR
Rh28	ALV RMS	<0.001	> 2.5	0	0.04	<0.001	MCR
Rh30	ALV RMS	<0.001	> 4.0	0.2	0.33	0.002	CR
Rh30R	ALV RMS	<0.001	> 3.2	0.2	0.18	<0.001	MCR
Rh18	EMB RMS	0.019	1.9	>4	0.67	0.063	PD2
BT-28	Medulloblastoma	0.234	1.1	>4	0.66	0.351	PD1
BT-45	Medulloblastoma	0.894	0.7	>4	1.14	0.604	PD1
BT-50	Medulloblastoma	0.571	> 1.2	4	0.78	0.604	PD1
BT-41	Ependymoma	0.006	1.5	>4	0.39	0.002	PD1
BT-44	Ependymoma	0.841	1	>4	0.92	0.739	PD1
GBM2	Glioblastoma	<0.001	1.8	>4	0.41	<0.001	PD2
BT-39	Glioblastoma	0.757	1	>4	0.96	0.853	PD1
D645	Glioblastoma	<0.001	> 5.7	0.4	0.28	<0.001	PR
D456	Glioblastoma	<0.001	> 2.9	3.6	0.26	0.004	CR
NB-SD	Neuroblastoma	<0.001	> 4.1	3.2	0.15	<0.001	CR
NB-1771	Neuroblastoma	<0.001	> 7.2	0	0.04	<0.001	MCR
NB-1691	Neuroblastoma	<0.001	> 4.1	0	0.1	<0.001	MCR
NB-EBc1	Neuroblastoma	<0.001	2.7	>4	0.23	0.002	PD2
NB-1643	Neuroblastoma	<0.001	> 7.3	0	0.08	<0.001	MCR
OS-1	Osteosarcoma	0.02	1.2	>4	0.74	0.011	PD1
OS-2	Osteosarcoma	0.225	1	>4	1.15	0.052	PD1
OS-17	Osteosarcoma	0.251	1	>4	1.02	0.631	PD1
OS-9	Osteosarcoma	0.144	1	>4	0.93	0.165	PD1
OS-33	Osteosarcoma	<0.001	1.3	>4	0.63	0.002	PD1
OS-31	Osteosarcoma	0.152	1.1	>4	0.74	0.063	PD1

Red shading in the p-value column indicates a significant difference in EFS distribution between treated and control groups. Shading in the EFS columns indicates xenografts that have either high (dark blue), intermediate (light blue), or indeterminate (gray) activity.

Gene Expression for selected genes that are markers for neuroendocrine origin (Affymetrix U133Plus2)

Rhabdomyosarcoma (blue boxes) and neuroblastoma (red boxes) xenografts and cell lines generally expressed neuroendocrine markers [NCAM1, synaptophysin (SYP), chromogranin A (CHGA)].



*Gray indicates an absent call from Affymetrix quality control

CONCLUSIONS

- NTX-010 demonstrated *in vitro* activity against Ewing sarcoma, alveolar rhabdomyosarcoma and neuroblastoma models.
- NTX-010 demonstrated high-level *in vivo* activity against models of rhabdoid and Wilms tumor of the kidney, alveolar rhabdomyosarcoma, glioblastoma and neuroblastoma.
- Ewing sarcoma showed no sensitivity *in vivo*, while demonstrating significant in vitro sensitivity.
- Four of 4 alveolar rhabdomyosarcoma and 4 of 5 neuroblastoma xenografts achieved CR or maintained CR.
- Further work is needed to determine the molecular characteristics associated with response.

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