In vitro: In vitro testing was performed using DIMSCAN, a semiautomated fluorescence-based digital image microscopy system that quantifies viable (using fluorescein diacetate [FDA]) cell numbers in tissue culture multiwell plates (Kang MN, et al. Pediatr Blood Cancer 56:239-249, 2011). Testing was for 96 hours at concentrations from 1.0 to 100 nM with replicates of 6-12 per data point. Data were analyzed by fitting a non-linear regression model-sigmoidal dose-response model to the response-relative fluorescence values vs. the concentration. In vivo: Standard PPTP methods for in vivo testing were employed (http://pptpChildrensHospital.org/METHODS/). NSC750854 was tested in vivo using a dose of 5 mg/kg administered by the intraperitoneal (IP) route daily for 5 days repeated at day 15. For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2–0.5 cm³. Two perpendicular tumor diameters were measured at either once or twice weekly intervals with digital vernier calipers. Assuming tumors to be spherical, volumes were calculated from the formula (π/6)d³, where d represents the mean diameter.

The primary activity measures were the objective response measure (see legend to figure at right) and the EFS T/C measure. The EFS T/C value is defined by the ratio of the median time to event of the treatment group and the median time to event of the respective control group.

### NSC750854 IN VIVO RESULTS AND CONCLUSIONS

- **NSC750854** was well tolerated (1.7% mortality) at the dose (5 mg/kg IP) and schedule (daily x 5 repeated at day 15) evaluated, and 30 of 30 xenograft models were considered evaluable for efficacy.
- **NSC750854** induced significant differences in EFS distribution compared to control in 26 of 30 (87%) evaluable solid tumor xenografts.
- **NSC750854** induced tumor growth inhibition meeting criteria for intermediate or high EFS T/C in 14 (47%) xenografts (48%), with this level of activity most consistently observed in the rhabdomyosarcoma panel (4 of 4).
- **NSC750854** induced objective responses in 11 of 30 (37%) of solid tumor models, including two glioblastoma models.
- While some of the xenografts that were responsive to **NSC750854** have also shown responsiveness to other cytotoxic agents (e.g., KT-19 and CHLA-258), other responsive models have shown limited sensitivity to most agents against which they’ve been tested (e.g., BT-29 and CHLA-79).
- **NSC750854** demonstrated a wide spectrum of antitumor activity. However, as with other agents, accurate translation of these results to the clinic will depend upon the tolerance of patients to the agent and upon how the drug exposures causing tumor regression in this study compare to those achievable in humans.

### IN VIVO ACTIVITY OF NSC750854 AGAINST PPTP SOLID TUMOR XENOGRAFT MODELS

**Cell line** | **Histology** | **EFS T/C** | **P-** | **T** | **P-** | **Response** |
--- | --- | --- | --- | --- | --- | --- |
BT-29 | Phaeochromocytoma | 1.5 | >0.001 | 0.00 | 0.00 | CR |
BT-19 | Retinoblastoma | 1.6 | >0.001 | 0.00 | 0.00 | CR |
BT-18 | Retinoblastoma | 1.5 | >0.001 | 0.00 | 0.00 | CR |
BT-10 | Retinoblastoma | 3.4 | <0.001 | 0.00 | 0.00 | CR |
BT-11 | Retinoblastoma | 3.4 | <0.001 | 0.00 | 0.00 | CR |
SK-NP-1 | Ewing | 0.9 | 0.050 | 1.39 | 0.00 | PD |
SK-NY-5 | Ewing | 1.3 | 0.072 | 0.76 | 0.00 | PD |
TC-71 | Ewing | 1.9 | <0.001 | 0.00 | 0.00 | PD |
NL-10 | Ataxia Telangiectasia | 3.8 | <0.001 | 0.00 | 0.00 | CR |
NL-24R | Ataxia Telangiectasia | 3.4 | <0.001 | 0.00 | 0.02 | PD |
NB-1691 | Neuroblastoma | 1.9 | <0.001 | 0.00 | 0.00 | CR |
NB-1771 | Neuroblastoma | 1.3 | 0.272 | 0.75 | 0.075 | PD |
NB-1692 | Neuroblastoma | 1.9 | <0.001 | 0.00 | 0.00 | PD |
NB-1773 | Neuroblastoma | 0.9 | 0.388 | 1.18 | 1.000 | PD |
OS-31 | Osteosarcoma | 1.3 | 0.212 | 0.39 | 1.000 | PD |
OS-33 | Osteosarcoma | 3.4 | <0.001 | 0.00 | 0.00 | CR |
OS-17 | Osteosarcoma | 2.1 | 0.241 | 0.73 | 0.29 | PD |
OS-9 | Osteosarcoma | 1.1 | 0.090 | 0.85 | 0.67 | PD |
D456 | Glioblastoma | 1.9 | <0.001 | 0.00 | 0.00 | CR |
D645 | Glioblastoma | 1.5 | 0.011 | 0.85 | 0.02 | PD |
GBM2 | Glioblastoma | 1.7 | 0.006 | 0.93 | 0.009 | PD |
Rh18 | Embryonal RMS | 3.2 | <0.001 | 0.00 | 0.00 | CR |
Rh30R | Alveolar RMS | 1.7 | <0.001 | 0.00 | 0.00 | CR |
SK-NEP-1 | Ewing | 0.9 | 0.388 | 1.18 | 1.000 | PD |
KT-16 | Wilms | 1.7 | <0.001 | 0.00 | 0.00 | CR |

**Legend:**
- **CR (Complete response):** Tumor volume <50% regression in tumor volume, <50% regression in tissue culture multiwell plates (Kang MN, et al. Pediatr Blood Cancer 56:239-249, 2011). Testing was for 96 hours at concentrations from 1.0 to 100 nM with replicates of 6-12 per data point.
- **PD (Partial response):** Assuming tumors to be spherical, volumes were calculated from the formula (π/6)d³, where d represents the mean diameter.
- **T/C** compares to those achievable in humans.