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Evaluation of Bortezomib against Childhood Tumor Models by the Pediatric Preclinical Testing Program (PPTP)

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Abstract

Previous studies have validated the use of xenografts derived from childhood cancers as models that can identify drugs known to be active against their respective clinical disease. Importantly, these models have correctly identified novel agents that have prospectively been shown to have significant activity in phase 1 and 2 clinical trials in children. Because cancer in children is relatively rare, and only limited numbers of clinical trials can be undertaken, a mechanism for identifying and prioritizing agents is required. The PPTP is a program established by NCI with a goal to identify novel agents that have significant activity against preclinical models of childhood cancers as one mechanism for prioritizing their clinical testing. We have established panels of xenograft models representing kidney tumors/rhabdoid tumors (8), sarcoma (10), non-glioblastoma brain tumors (8), glioblastoma (6), neuroblastoma (8), osteosarcoma (8) and acute lymphocytic leukemia (10) as a primary screen to identify novel agents. Tumors were selected based upon their growth characteristics and upon gene expression profiles similar to their respective clinical counterparts. Models have also been selected to represent tumors and histologies that have poor prognosis (e.g., rhabdoid tumors, GBM). 25/58 tumor models were established from previously treated patients. Approximately 12 agents per year can be evaluated by the PPTP. To 'calibrate' the system we first evaluated vincristine, a standard cytotoxic agent used as a component of multiple protocols. Vincristine (1 mg/kg q7d x 6 weeks) elicited objective regressions in 10/35 solid tumor models and 7/8 ALL models. Among the solid tumor lines, responses to vincristine were noted in 1/6 kidney tumors/rhabdoid tumors, 3/6 rhabdomyosarcoma, 0/2 Ewing sarcoma, 0/1 ependymoma, 2/5 medulloblastoma, 1/4 glioblastoma, 0/6 neuroblastoma, and 3/6 osteosarcoma. We next evaluated the novel proteasome inhibitor bortezomib (Velcade, PS-341) at 1 mg/kg twice weekly x 6 weeks. Accumulating data show no responses in 33 solid tumors (kidney, sarcoma, brain tumors, neuroblastoma, and osteosarcoma). Three of 6 ALL models showed complete responses to bortezomib, suggesting that bortezomib may warrant further evaluation for childhood ALL.

PPTP Tumor Panels and Test Sites

- **Rhabdomyosarcoma (n=5) and Ewing sarcoma (n=3)**
  - **Dr. Peter Houghton (St. Jude Children's Research Hospital)**
- **Neuroblastoma (n=6)**
  - **Dr. John Maris (Children's Hospital of Philadelphia)**
- **Osteosarcoma (n=6)**
  - **Dr. Richard Gorlick (Albert Einstein College of Medicine)**
- **Acute lymphoblastic leukemia (n=8)**
  - **Dr. Richard Lock (Children's Cancer Institute Australia)**
- **Brain tumors, Glioblastoma (n=4)**
  - **Dr. Henry Friedman (Duke University Medical Center)**
- **Brain tumors, Non-glioblastoma (n=6)**
  - **Dr. Peter Houghton (St. Jude Children's Research Hospital)**
- **Kidney cancers (n=6)**
  - **Dr. Peter Houghton (St. Jude Children's Research Hospital)**
- **In vitro panel (n=23)**
  - **Dr. Patrick Reynolds (Children's Hospital of Los Angeles)**

Gene expression profiles and chromosome copy number abnormalities are being determined for each of the PPTP lines. These data will be publicly available.

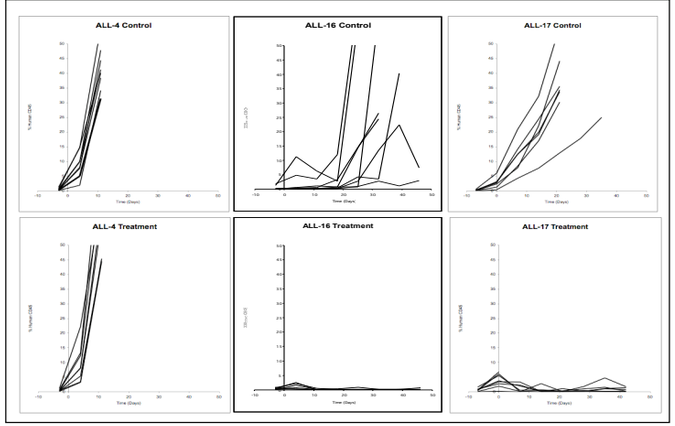
Brain Tumor Panel (glioblastoma)	Duke University School of Medicine
SJ-GBM2	Glioblastoma
SJ-BT39	Glioblastoma
D645	Glioblastoma
D456	Glioblastoma
SJ-BT56 (extended panel)	Glioblastoma
D212 (extended panel)	Glioblastoma
Brain Tumor Panel (non-glioblastoma)	**St. Jude Children's Research Hospital**
BT-28	Medulloblastoma (diagnosis)
BT-31	Anaplastic medulloblastoma (diagnosis)
BT-32	Medulloblastoma (diagnosis)
BT-45	Medulloblastoma (diagnosis)
BT-36	Anaplastic Ependymoma (diagnosis)
BT-41	Ependymoma (relapse)
BT-46 (extended panel)	Medulloblastoma (diagnosis)
BT-50 (extended panel)	Medulloblastoma (diagnosis)
Sarcoma Panel	**St. Jude Children's Research Hospital**
EW1	Ewing sarcoma (relapse)
EW5	Ewing sarcoma (diagnosis)
EW8	Ewing sarcoma
RH10	Rhabdomyosarcoma, alveolar (relapse)
RH18*	Rhabdomyosarcoma, embryonal (diagnosis)
RH28	Rhabdomyosarcoma, alveolar (diagnosis)
RH30*	Rhabdomyosarcoma, alveolar (diagnosis)
RH41*	Rhabdomyosarcoma, alveolar (relapse)
RH36 (extended panel)	Rhabdomyosarcoma, embryonal (relapse)
RH65 (extended panel)	Rhabdomyosarcoma, alveolar (relapse)
Osteosarcoma panel	**Albert Einstein College of Medicine**
OS-1	Osteosarcoma (primary/untreated)
OS-2	Osteosarcoma (primary/untreated)
OS-17	Osteosarcoma (primary/untreated)
OS-164	Osteosarcoma (primary/previously treated)
OS-166	Osteosarcoma (osteoblastic primary/relapse)
OS-187	Osteosarcoma (primary/untreated)
OS-21 (extended panel)	Osteosarcoma (primary/untreated)
OS-160 (extended panel)	Osteosarcoma (lung metastasis/relapse)
Acute Lymphoblastic Leukemia panel	**Children's Cancer Institute of Australia**
ALL-2	c-ALL /Relapse 3 (CR1 = 30 mos)
ALL-3	Pre-B ALL /Diagnois (CR1 = 38 mos)
ALL-4	Ph+ ALL /Diagnosis (CR1 = 10 mos)
ALL-7	Biphenotypic /Diagnosis (CR1 = 7 mos)
ALL-8	T-ALL /Relapse 1 (CR1 = 17 mos)
ALL-16	T-ALL /Diagnosis (CR1 = 103+ mos)
ALL-17	c-ALL /Diagnosis (CR1 = 25 mos)
ALL-19	c-ALL /Relapse 1 (CR1 = 4 mos)
ALL-10 (extended panel)	c-ALL /Diagnosis (CR1 = 57+ mos)
ALL-11 (extended panel)	c-ALL /Diagnosis (CR1 = 120+ mos)
Neuroblastoma panel	**Children's Hospital of Philadelphia**
NB-SD	MYCN amplified (previously treated)
NB-1771	MYCN amplified (diagnosis)
NB-1691	MYCN amplified (relapse)
NB-EBc1*	Not MYCN amplified (relapse)
CHLA-79	Not MYCN amplified (relapse)
NB-1643*	MYCN amplified (diagnosis)
NB-1382 (extended panel)	MYCN amplified (relapse)
SK-N-AS (extended panel)	Not MYCN amplified (diagnosis)
Kidney/rhabdoid tumor panel	**St. Jude Children's Research Hospital**
BT-29	Atypical teratoid rhabdoid (CNS) (diagnosis)
WT-14	Rhabdoid, kidney (relapse)
WT-11	Wilms favorable histology (diagnosis)
WT-13	Wilms diffuse anaplastic (diagnosis)
WT-16	Rhabdoid, kidney (relapse)
SKNEP	Wilms diffuse anaplastic (relapse)
WT-10 (extended panel)	Wilms favorable histology (diagnosis)
WT-12 (extended panel)	Rhabdoid, kidney (diagnosis)

Methods for PPTP in Vivo Testing

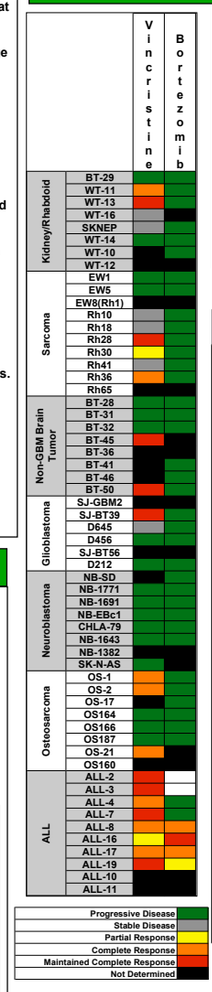
Stage 1 testing involves testing an agent across the entire PPTP panel of childhood cancer xenograft lines at its MTD (or at a dose selected based on PK/PD studies using adult preclinical models). If activity is observed in Stage 1 testing, then further testing in Stage 2 can address, as appropriate, the dose-response curve for antitumor activity, PK/PD studies, and drug combination studies. Procedures for Stage 1 testing are provided below.

- **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 either once or twice 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.**
- **Bortezomib was provided by Millennium Pharmaceuticals through Cancer Therapy Evaluation Program (NCI). Bortezomib was dissolved in DMSO(2%final)/saline & administered IP, twice weekly x 6 weeks at a dose of 1 mg/kg.**
- **Vincristine was provided by the Developmental Therapeutics Program, NCI. Vincristine was dissolved in saline and administered IP weekly x 6 weeks at a dose of 1 mg/kg.**
- **Solid Tumor Response Criteria:**
  - **Progressive Disease (PD) = greater than 25% increase over starting volume.**
  - **Stable Disease (SD) = not greater than 25% increase over starting volume, but not more than 50% volume regression.**
  - **Partial response (PR) = volume regression of 50%, but with measurable tumor (≥ 0.10 cm3) at all times.**
  - **Complete response (CR) = disappearance of measurable tumor mass (<0.10 cm3) at any point within 6 weeks after initiation of therapy.**
  - **Maintained complete response (MCR) = CR without tumor re-growth in 6-wk study period.**
- **Leukemia Response Criteria:**
  - **PD = CD45% never drops below 1%, events before 42d**
  - **SD = CD45% never drops below 1%, no events before 42d**
  - **PR = CD45% < 1% for only 1 week (+/- events before 42d)**
  - **CR = CD45% < 1% for ≥ 2 weeks (+/- events before 42d)**
  - **MCR = <1% at 42d**

Bortezomib Activity against ALL Lines



Bortezomib & Vincristine Activity: PPTP in Vivo Lines



***Bortezomib antitumor activity*:**

- **No objective responses or stable disease in solid tumor lines**
- **ALL lines (n=6): 1 MCR and 2 CR**

***Vincristine antitumor activity*:**

- **Responses in multiple solid tumors, including:**
  - **Wilms tumor (n=4): 1CR & 1MCR**
  - **Rhabdomyosarcoma (n=6): 1MCR, 1CR & 1 PR**
  - **Osteosarcoma (n=6): 3CR**
  - **Glioblastoma (n=4): 1MCR**
  - **Medulloblastoma (n=5): 2CR**
- **No objective responses for neuroblastoma lines (n=6)**
- **ALL lines (n=8): 4MCR and 3CR**

DISCUSSION & CONCLUSIONS

- **Stage 1 testing results for bortezomib suggest that this agent warrants further evaluation for pediatric ALL. Data from one additional ALL line will be included in the final Stage 1 report.**
- **Activation of NFκB is reported to occur commonly in pediatric ALL, providing a potential mechanism for bortezomib activity in this disease. The combination of bortezomib, dexamethasone, & doxorubicin has been evaluated in adults with myeloma and there is interest in evaluating this combination in children with ALL.**
- **Stage 1 testing of bortezomib provides no leads for the use of bortezomib in children with solid tumors.**
- **The antitumor activity of vincristine observed against the ALL, rhabdomyosarcoma, and Wilms tumor lines was expected, given the activity of these agents in children with these tumors.**
- **The responses to vincristine observed against the osteosarcoma lines were unexpected. Though vincristine has been used in treating children with osteosarcoma, its single agent activity is considered to be limited.**
- **Studies are in progress to identify associations between the gene expression profiles of the xenograft lines and their responsiveness to bortezomib and vincristine. Patterns of gene expression associated with activity could be clinically relevant, if similar patterns are present in patient specimens.**
- **This initial PPTP experience demonstrates the feasibility of timely testing of agents across a relatively large panel of childhood cancer xenograft lines, with one agent initiating testing each month. Future agents to be tested will address molecular targets such as Src family kinases, VEGF receptor, histone deacetylase, ErbB family kinases, mTOR, and Bcl-2 family inhibitors of apoptosis.**

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