#5265 Pediatric Preclinical Testing Program (PPTP) Stage 1 Evaluation of BMS-754807 IGF-1 Receptor Inhibitor

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Abstract

Background: Signaling through the type-1 insulin-like growth factor receptor (IGF-1R) is involved in autocrine or paracrine growth of many tumor types including childhood malignancies, and provides a strong anti-apoptotic signal that induces resistance to many forms of cellular stress. BMS-754807 is a potent inhibitor of IGF-1R and the insulin receptor that has entered phase 1 clinical trials.

Methods: The PPTP includes a molecularly characterized *in vitro* panel of cell lines (n=27) and *in vivo* panel of xenografts (n=61) representing common types of childhood solid tumors and ALL. BMS-754807 was tested against the PPTP *in vitro* panel at concentrations from 1.0 nM to 10 μM and results were compared to those obtained with the anti-IGF-1R antibody mAb391 (50 μg/ml). *In vivo* testing used a dose of 25 mg/kg BID administered daily x 6 x 6 weeks by oral gavage. *In vivo* antitumor activity was primarily assessed by using response criteria modeled after the clinical setting and by using a time to event measure based on the median 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: The median EC50 for BMS-754807 against the *in vitro* panel was 0.62 μM (range, 0.07 – 4.96 μM). The median BMS-754807 EC50 value for the 5 cell lines with the greatest response to mAb391 was 0.12 μM compared to 1.1 μM for the 11 cell lines with the least response to mAb391 (p=0.0009). These results are consistent with a specific IGF-1R effect that has half-maximal response in the 0.1 μM range and with a non-IGF-1R effect that shows half-maximal response at approximately 1 μM. The mortality rate among treated mice was 6.5%, and 39 of 45 xenograft models were evaluable for efficacy. BMS-754807 induced significant differences in EFS distribution compared to controls in 18 of 32 evaluable solid tumor xenografts (56%) tested, but in none of the ALL xenografts studied. Criteria for intermediate activity for the time to event activity measure (EFS T/C > 2) were met in 7 of 27 solid tumor xenografts and were most commonly observed in the neuroblastoma (3 of 6) and rhabdomyosarcoma (2 of 6) panels. Objective responses (i.e., tumor regression) were not observed for any xenografts. The best response was PD2 (progressive disease with growth delay), which was observed in two or more xenografts in the rhabdomyosarcoma, neuroblastoma, osteosarcoma, Ewing, and Wilms tumor panels.

Conclusions: BMS-754807 showed broad tumor growth inhibition activity against the PPTP *in vivo* preclinical models. Future studies will focus on defining how pharmacokinetic and pharmacodynamic effects of BMS-754807 relate to tumor sensitivity and to evaluating combinations of BMS-754807 with standard cytotoxic agents.

PPTP *in Vitro* & *in Vivo* Testing Methods

In vitro: *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 1.0 nM to 10 μM with replicates of 6-12 per data point. The activity of BMS-754807 was compared to that of a murine monoclonal antibody (mAb391) directed against the human IGF-1 receptor tested at a saturating concentration (50 μg/ml) Data were analyzed by fitting to a non-linear regression model-sigmoidal dose-response model.

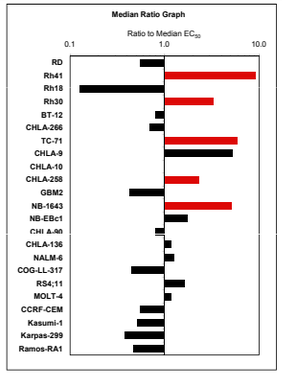
In vivo: Standard PPTP methods for in vivo testing were employed (see http://pptp.nchresearch.org/documents/detailedAnalysisMethods.pdf). BMS-754807 was administered P.O. twice daily for 6 days per week for 6 consecutive weeks at 25 mg/kg.

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 (π/6)×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.

BMS-754807 *IN VITRO* ACTIVITY

- The median EC_{50} for the in vitro panel was 0.62 μM.
- There was > 70-fold range in EC_{50} values, with the most sensitive cell line being the rhabdomyosarcoma cell line Rh41 (EC_{50} 0.07 μM) and the least sensitive cell line being Rh18 (EC_{50} 4.96 μM).
- The median EC_{50} for the 4 Ewing sarcoma cell lines was less than that for the remaining 19 PPTP cell lines (0.19 μM versus 0.78 μM, p=0.0470).



- The median EC_{50} value for BMS-754807 for the 5 cell lines with the greatest response to the anti-IGF-1R monoclonal antibody mAb391 (highlighted in red bars in the figure) was 0.12 μM,
- The median EC_{50} for the 10 cell lines with the least evidence of mAb391 treatment effect was approximately 10-fold higher at 1.0 μM (p=0.0017).
- This observation is consistent with a specific IGF-1R effect for BMS-754807 that has half-maximal response in the 0.1 μM range and that is observed in a minority of the PPTP cell lines, and with a non-IGF-1R effect that occurs in all of the cell lines and that shows half-maximal response at approximately 1 μM.

BMS-754807 was provided for testing by Bristol-Myers Squibb
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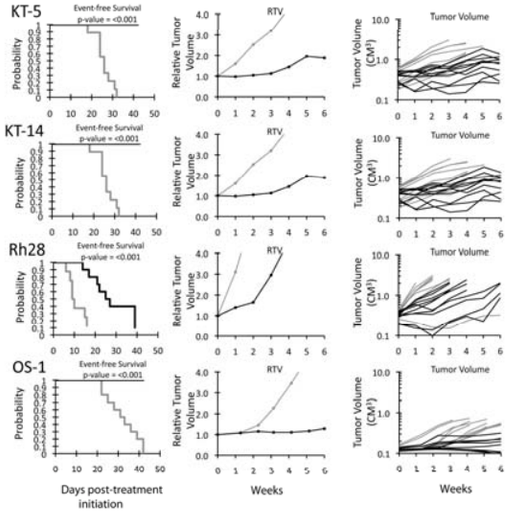
BMS-754807 *IN VIVO* ACTIVITY

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	Overall Group Response	Heat Map
BT-29	Rhabdoid	0.027	1.5	>4	0.64	0.035	PD1	PD1
KT-14	Rhabdoid	<0.001	> 1.6	1.9	0.35	<0.001	PD2	PD2
KT-12	Rhabdoid	0.008	1.5	>4	0.75	0.035	PD1	PD1
KT-11	Wilms	0.004	1.8	>4	0.51	0.001	PD2	PD2
KT-13	Wilms	<0.001	1.5	>4	0.39	<0.001	PD1	PD1
KT-5	Wilms	<0.001	2.1	>4	0.60	0.003	PD2	PD2
SK-NEP-1	Ewing	0.231	1.1	>4	0.86	0.218	PD1	PD1
EW5	Ewing	0.042	2.1	>4	0.48	0.017	PD2	PD2
EW8	Ewing	0.006	1.8	>4	0.73	0.035	PD2	PD2
TC-71	Ewing	0.126	0.9	>4	1.15	0.353	PD1	PD1
Rh10	ALV RMS	0.979	1.7	>4	0.50	0.043	PD2	PD2
Rh28	ALV RMS	0.203	2.6	>4	0.51	0.009	PD2	PD2
Rh30	ALV RMS	0.429	1.1	>4	0.81	0.105	PD1	PD1
Rh30R	ALV RMS	<0.001	2.3	>4	0.34	<0.001	PD2	PD2
Rh41	ALV RMS	0.121	1.5	>4	0.59	0.011	PD1	PD1
Rh18	EMB RMS	<0.001	2.1	>4	0.38	<0.001	PD2	PD2
BT-28	Medulloblastoma	0.504	0.9	>4	0.96	0.912	PD1	PD1
BT-45	Medulloblastoma	0.174	0.9	>4	1.10	0.280	PD1	PD1
BT-41	Ependymoma	1.000	.	2.4	0.71	0.089	PD2	PD2
BT-44	Ependymoma	0.301	1.1	>4	0.71	0.029	PD1	PD1
NB-SD	Neuroblastoma	0.934	0.9	>4	1.10	0.574	PD1	PD1
NB-1771	Neuroblastoma	<0.001	2.5	>4	0.30	0.002	PD2	PD2
NB-1691	Neuroblastoma	0.426	1.0	>4	0.88	0.481	PD1	PD1
NB-EBc1	Neuroblastoma	<0.001	2.7	>4	0.27	<0.001	PD2	PD2
NB-1643	Neuroblastoma	0.012	3.4	>4	0.52	0.200	PD2	PD2
SK-N-AS	Neuroblastoma	0.004	1.6	>4	0.59	0.007	PD2	PD2
OS-1	Osteosarcoma	<0.001	> 1.3	1.3	0.75	0.035	PD2	PD2
OS-2	Osteosarcoma	0.055	> 1.2	3.0	0.76	0.079	PD2	PD2
OS-17	Osteosarcoma	0.011	> 1.4	3.1	0.77	0.074	PD2	PD2
OS-9	Osteosarcoma	<0.001	1.6	>4	0.64	<0.001	PD2	PD2
OS-33	Osteosarcoma	0.002	1.3	>4	0.74	0.003	PD1	PD1
OS-31	Osteosarcoma	0.477	1.1	>4	0.94	0.353	PD1	PD1
ALL-2	ALL B-precursor	0.612	0.7	>25	.		PD1	PD1
ALL-3	ALL B-precursor	0.167	0.5	>25	.		PD1	PD1
ALL-7	ALL B-precursor	0.932	1.0	>25	.		PD1	PD1
ALL-8	ALL T-cell	0.627	0.9	>25	.		PD1	PD1
ALL-16	ALL T-cell	0.141	0.5	>25	.		PD1	PD1
ALL-17	ALL B-precursor	0.100	0.6	>25	.		PD1	PD1
ALL-19	ALL B-precursor	0.097	0.7	>25	.		PD1	PD1

- Red shading in the p-value columns indicates a significant difference in EFS distribution or Tumor Volume T/C 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.
- **PD1 (Progressive Disease 1):** >25% ↑ in tumor volume, TGD value ≤1.5; **PD2 (Progressive Disease 2):** >25% ↑ in tumor volume, TGD value >1.5; **SD (Stable Disease):** <25% ↑ in tumor volume, <50% regression

Controls (gray lines)
Treated (black lines)



IN VIVO RESULTS AND CONCLUSIONS

- BMS-754807 was evaluated in 45 xenograft models at 25 mg/kg BID using a 6 days per week x 6 weeks schedule. BMS-754807 was tolerated at this dose, with mortality for treated animals of 6.5%.
- BMS-754807 induced significant differences in EFS distribution compared to controls in 18 of 32 evaluable solid tumor xenografts (56%). The tested ALL xenografts did not show significant treatment effects to BMS-754807.
- Objective responses were not observed for any solid tumor and ALL xenografts.
- Criteria for intermediate activity for the time to event activity measure (EFS T/C > 2) were met in 7 of 27 (26%) evaluable solid tumor xenografts.
- Intermediate EFS T/C activity was most commonly observed in the neuroblastoma (3 of 6) and rhabdomyosarcoma panels (2 of 6). Single xenografts in the Wilms tumor and Ewing sarcoma panels also showed intermediate activity.
- The broad activity of BMS-754807 in pediatric sarcomas and neuroblastoma xenografts suggests that this agent may be effective for selected pediatric cancers. Combinations targeting multiple, related signaling pathways warrant evaluation.