Abstract A212

Pediatric Preclinical Testing Program (PPTP) Evaluation of the Fully Human Anti-IGF-1R Antibody SCH 717454

Peter J. Houghton[1], John M. Maris[2], Henry S. Friedman[3], Stephen T. Keir[3], Richard B. Lock[4], Hernan Carol[4],Richard Gorlick[5], E. Anders Kolb[6], Nino Keshelava[7], C. Patrick Reynolds[7], Christopher Morton[1], Malcolm A. Smith[8].
[1]St. Jude Children's Research Hospital, [2]Children's Hospital of Philadelphia, [3]Duke University, [4]Children's Cancer Inst., Australia, [5]Albert Einstein College of Medicine, [6]A.I. duPont Hospital for Children, [7]Children's Hospital of Los Angeles, [8]CTEP/NCI.

Abstract

Background: SCH 717454 is a fully human antibody directed against the insulin-like growth factor 1 receptor (IGF-1R), which is implicated in the growth and metastatic phenotype of a broad range of malignancies. IGF-1R signaling may be of particular importance in the childhood cancer setting, with preclinical data supporting its role in the growth and survival of a number of pediatric cancers, including of neuroblastoma, Ewing sarcoma, rhabdomyosarcoma, Wilms tumor, and osteosarcoma. The activity of SCH 717454 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 ALL. SCH 717454 was tested against the PPTP in vitro panel at concentrations ranging from 0.01nM to 100 nM and was tested against the PPTP in vivo panel at a dose of 0.5 mg per mouse administered twice weekly for four weeks via intraperitoneal 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 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: SCH 717454 was ineffective at retarding growth of cell lines in the in vitro panel. SCH 717454 significantly increased event-free survival in 20 of 35 (57%) solid tumor xenograft models with tumor regressions in one Ewing sarcoma model (complete response) and 2 osteosarcoma models (maintained complete responses). Although objective responses were not noted in the neuroblastoma panel, tumor growth was well-controlled during the 4 weeks of SCH 717454 treatment for 3 of 6 evaluable neuroblastoma xenografts. Using the time to event activity measure, SCH 717454 had intermediate (n=9) or high (n=1) activity against 31 evaluable solid tumor xenografts, including xenografts from the rhabdoid tumor (1 of 3), Ewing (2 of 5), rhabdomyosarcoma (1 of 4), glioblastoma (1 of 4), ependymoma (1 of 1), neuroblastoma (2 of 5), and osteosarcoma panels (2 of 4). SCH 717454 showed little activity against the 8 xenografts of the acute lymphoblastic leukemia panel.

Conclusions: SCH 717454 showed little in vitro activity against the PPTP's in vitro panel, which may be related to the use of 20% fetal bovine serum and insulin in the growth media used for testing. SCH 717454 demonstrated broad antitumor activity against the PPTP's in vivo solid tumor panels. Further characterization of the molecular predictors of response and of the activity of combinations of SCH 717454 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 0.01 nM to 0.1 μM 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.

SCH 717454 *In Vitro* Activity

- SCH 717454 showed little evidence for drug effect against the 23 cell lines of the PPTP *in vitro* panel.
- The median growth inhibition for the *in vitro* panel at the highest concentration tested was only 5%.
- The maximal growth inhibition achieved was only 30% and was observed for the T-cell ALL line, MOLT-4.

Printed by Biomedical Communications

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 (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.

Drug: SCH 717454 was provided to the PPTP by Schering-Plough Research Institute. SCH 717454 was dissolved in 20mM sodium acetate pH5 buffer containing 150mM sodium chloride, and administered intraperitoneally twice weekly for 4 consecutive weeks at a recommended dose of 0.5 mg per animal. SCH 717454 was provided to each testing site in coded vials for blinded testing according the PPTP's standard operating procedures.

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.

SCH 717454 *In Vivo* Activity

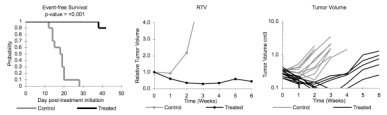
- SCH 717454 induced significant differences in EFS distributions compared to controls in 20/35 solid tumor models and 2/8 ALL models.
- Among 31 solid tumor xenografts evaluable for the time to event activity measure (EFS T/C), a Ewing tumor xenograft (EW5) met the criteria for high activity with an EFS T/C value of >2.3 and with a final tumor volume less than its starting tumor volume.
- Two osteosarcoma xenografts showed continuous tumor regression during the 4 weeks of treatment and 2 subsequent weeks of observation (OS-1 and OS-9).

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	Overall Group Response
BT-29	Rhabdoid	0.066	1.4	>4	0.77	0.247	PD1
KT-16	Rhabdoid	0.24	1.2	>4	0.5	0.130	PD1
KT-14	Rhabdoid	<0.001	> 3.3	1.8	0.6	0.009	PD2
KT-10	Wilms	0.001	1.3	>4	0.43	<0.001	PD1
KT-11	Wilms	0.547	1	>4	0.99	1.000	PD1
KT-13	Wilms	0.374	1.1	>4	0.84	0.236	PD1
SK-NEP-1	Ewings	0.428	0.9	>4	1.08	0.829	PD1
EW5	Ewings	<0.001	> 2.3	0.4	0.12	<0.001	CR
EW8	Ewings	0.579	1.5	>4	0.86	0.243	PD1
TC-71	Ewings	0.246	1.2	>4	0.88	0.579	PD1
CHLA258	Ewings	<0.001	4.2	>4	0.29	<0.001	PD2
Rh28	ALV Rhabdomyosarcoma	<0.001	> 2.6	2.9	0.36	0.001	PD2
Rh30R	ALV Rhabdomyosarcoma	<0.001	1.8	>4	0.49	0.004	PD2
Rh41	ALV Rhabdomyosarcoma	<0.001	1.1	>4	0.65	0.065	PD1
Rh18	EMB Rhabdomyosarcoma	0.44	1	>4	0.74	0.353	PD1
BT-28	Medulloblastoma	0.121	1.2	>4	1.18	0.529	PD1
BT-46	Medulloblastoma	0.009	1.2	>4	0.69	0.011	PD1
BT-50	Medulloblastoma	0.474	.	1.4	0.94	0.436	PD2
BT-44	Ependymoma	<0.001	2.6	>4	0.66	0.003	PD2
GBM2	Glioblastoma	0.005	1.4	>4	0.73	0.007	PD1
BT-39	Glioblastoma	0.377	1.1	>4	0.87	0.631	PD1
D645	Glioblastoma	0.007	2.2	>4	0.58	<0.001	PD2
D456	Glioblastoma	0.008	1.1	>4	0.77	0.008	PD1
NB-SD	Neuroblastoma	<0.001	> 1.8	3.7	0.41	0.006	PD2
NB-1771	Neuroblastoma	0.595	1.1	>4	0.64	0.063	PD1
NB-1691	Neuroblastoma	0.025*	0.9	>4	1.34	0.075	PD1
NB-EBc1	Neuroblastoma	0.003	> 2.0	1.4	0.8	0.043	PD2
CHLA-79	Neuroblastoma	0.003	2.1	3.9	0.69	0.123	PD2
NB-1643	Neuroblastoma	0.005	1.8	>4	0.53	0.005	PD2
OS-1	Osteosarcoma	<0.001	> 1.3	0.1	0.16	<0.001	MCR
OS-2	Osteosarcoma	<0.001	2.3	>4	0.5	<0.001	PD2
OS-17	Osteosarcoma	<0.001	2	>4	0.55	0.089	PD2
OS-9	Osteosarcoma	0.003	> 1.2	0.4	0.31	<0.001	MCR
OS-33	Osteosarcoma	0.587	0.9	>4	0.79	0.529	PD1
OS-31	Osteosarcoma	0.461	1.3	>4	0.88	0.274	PD1
ALL-2	ALL B-precursor	0.095*	0.7	>25	.		PD1
ALL-3	ALL B-precursor	0.743	0.4	>25	.		PD1
ALL-4	ALL B-precursor	0.194	0.8	>25	.		PD1
ALL-7	ALL B-precursor	0.544	1	>25	.		PD1
ALL-8	ALL T-cell	0.408	1.5	>25	.		PD1
ALL-16	ALL T-cell	0.588	1	>25	.		PD1
ALL-17	ALL B-precursor	<0.001	3.1	>25	.		PD2
ALL-19	ALL B-precursor	0.009	1.5	>25	.		PD1

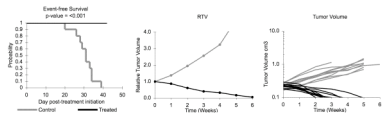
- Red shading in the p-value columns indicates a statistically significant difference between treated and control groups.
- Shading in the EFS columns indicates xenografts that have either high (dark blue), intermediate (light blue), or indeterminate (gray) activity.

SCH 717454 *In Vivo* Activity

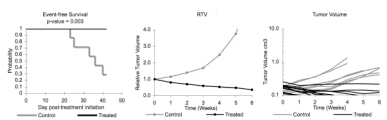
EW5



OS-1

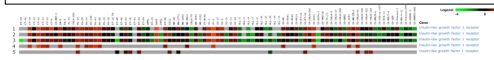


OS-9



IGF-1R Expression

- IGF-1R expression was evaluated using Affymetrix U133 Plus2 Arrays.
- 5 xenografts with very low IGF-1R expression did not respond (i.e., PD1) to SCH 717454. For example, OS-33 had very low expression and was one of two osteosarcoma xenografts that showed no response to SCH 717454.
- The 3 xenografts with CR or MCR responses had high IGF-1R expression.
- Some xenografts with moderate to high IGF-1R expression did not respond to SCH 717454 (e.g., Rh41).



CONCLUSIONS

- The activity of SCH 717454 as a single agent in the PPTP panel suggests a potential for clinical utility for selected pediatric solid tumors.
- SCH 717454 was able to control tumor growth in approximately one-third of the solid tumor xenografts, but overall the objective response rate to SCH 717454 was modest with 3 complete responses.
- Potential clinical applications for IGF-1R inhibitors will include rationally designed combinations.
- Further testing through the PPTP will help to identify combination treatment strategies maximizing IGF-1R inhibition with other molecular-targeted agents.

Supported by NCI NO1CM42216