Abstract B118

Pediatric Preclinical Testing Program (PPTP)
Evaluation of the EGFR and ErbB2 Inhibitor Lapatinib

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Abstract

Background: Lapatinib is a small molecule reversible tyrosine kinase inhibitor of EGFR and ErbB2 that shows in vitro and in vivo activity against a range of EGFR- and ErbB2-dependent adult cancer cell lines and that has clinical efficacy against ErbB2-overexpressing breast cancer. Lapatinib was studied by the PPTP to develop data concerning the relevance of EGFR family members as therapeutic targets for childhood cancers.
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. Lapatinib in vitro testing used media containing 20% FCS and evaluated concentrations from 1.0 nM to 10 μM, with viable cell numbers for treated and control replicates evaluated at 96 hours using the DIMSCAN fluorescence-based method. Lapatinib was tested against the PPTP in vivo panels using a twice-daily oral administration schedule for six weeks (5-days on, 2-days off) at a dose of 160 mg/kg (320 mg/kg/day). 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: EGFR and/or ErbB2 were expressed at detectable levels in most of the PPTP's cell lines and xenografts at the RNA level based upon data from Affymetrix U133 Plus 2.0 arrays. The median IC_{50} value for lapatinib against the entire PPTP cell line panel was 7.76 μM. IC_{50} values ranged from a low of 4.23 μM (CHLA-9, Ewing) to a maximum exceeding 10.0 μM in eight cell lines. Lapatinib was well tolerated in vivo, with toxicity in only 1.5% of the treated animals. Lapatinib induced significant differences in EFS distribution compared to controls in 1 of 41 evaluable xenografts tested. No xenografts met the criteria for intermediate activity for the PPTP EFS activity measure (EFS T/C value > 2.0 and a significant difference in EFS distribution). No objective responses were observed in any of the solid tumor panels or in the ALL panel. The best response observed was a single example of PD2 (progressive disease with growth delay).
Conclusions: The response of the PPTP cell lines to lapatinib corresponds to the pattern of response described previously for adult cancer cell lines that do not overexpress EGFR or ErbB2 and that have IC50 values exceeding 1 μM. Thus, the lapatinib in vitro activity against the PPTP cell lines likely represents "off-target" kinase inhibition effects. The lack of in vivo activity for lapatinib is consistent with previous reports that EGFR mutation and ErbB2 amplification are uncommon for childhood cancers. These results do not preclude a role for lapatinib in a biological subtype of a pediatric cancer that is not represented within the PPTP panel. However, when combined with preclinical and clinical experience to date for EGFR small molecule inhibitors, the results do suggest a limited role for EGFR family members as therapeutic targets for childhood cancers. (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 nM to 10 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.**

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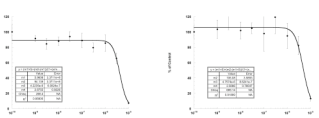
Lapatinib *in Vitro* Activity

- **The PPTP cell lines had similar patterns of response to lapatinib, with activity observed almost exclusively at concentrations exceeding 1 μM.**
- **Examples of dose response curves illustrating this general pattern of response are shown below for the Ewing sarcoma cell lines CHLA-9 (left) and CHLA-10 (right).**



- **The median IC_{50} value for lapatinib against the entire PPTP cell line panel was 7.76 μM. IC_{50} values ranged from a low of 4.23 μM to a maximum exceeding 10 μM in eight cell lines.**

Name	Diagnosis	IC_{50} (μM)
RD	Rhabdomyosarcoma	>10
Rh41	Rhabdomyosarcoma	7.76
Rh18	Rhabdomyosarcoma	>10
Rh30	Rhabdomyosarcoma	7.61
BT-12	Rhabdoid	7.70
CHLA-266	Rhabdoid	>10
TC-71	Ewing sarcoma	4.39
CHLA-9	Ewing sarcoma	4.23
CHLA-10	Ewing sarcoma	4.98
CHLA-258	Ewing sarcoma	>10
GBM2	Glioblastoma	6.06
NB-1643	Neuroblastoma	>10
NB-EBc1	Neuroblastoma	>10
CHLA-90	Neuroblastoma	>10
CHLA-136	Neuroblastoma	>10
NALM-6	Pre-B cell ALL	5.72
COG-LL-317	T-cell ALL	7.21
RS4;11	Pre-B cell ALL	8.64
MOLT-4	T-cell ALL	5.28
CCRF-CEM	T-cell ALL	7.71
Kasumi-1	AML	8.76
Karpas-299	Anaplastic Large Cell Lymphoma	9.30
Ramos-RA1	Burkitt's Lymphoma	6.28
MEDIAN		7.76
MINIMUM		4.23
MAXIMUM		>10

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: Lapatinib was provided to the PPTP by GlaxoSmithKline. Lapatinib was dissolved in 0.5% Hydroxypropylmethylcellulose (HPMC) with 0.1% Tween 80 in water and administered orally at a dose of 160 mg/kg twice daily for 5 days (2 days off), repeating for 6 weeks . Lapatinib was distributed 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.

Lapatinib *in Vivo* Activity

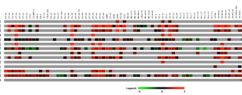
- **Lapatinib was well tolerated *in vivo* using a twice-daily oral administration schedule for 6 weeks (5-days on, 2-days off). Toxicity was observed in only 1.5% of treated animals, compared to 0.3% of animals in the control groups.**
- **Lapatinib induced significant differences in EFS distribution compared to controls in 1 of 41 evaluable xenografts tested, the medulloblastoma xenograft BT-28.**
- **No objective responses were observed in any of the solid tumor panels or in the ALL panel. The best response observed was a single example of PD2 (progressive disease with growth delay), which was for the alveolar rhabdomyosarcoma xenograft Rh41.**
- **The very limited activity is unlikely to be the result of inadequate drug exposure, as the lapatinib dose and schedule used are similar to the lapatinib dose and schedule that produced profound effects on tumor growth for ErbB2- and EGFR-driven adult cancer xenografts (Rusnak, et al. Mol Cancer Ther 2001:85-94).**

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	Overall Group Response
BT-29	Rhabdoid	0.241	1.3	>4	0.62	0.009	PD1
KT-16	Rhabdoid	0.93	1	>4	0.98	0.912	PD1
KT-14	Rhabdoid	0.494	1.1	>4	0.97	0.211	PD1
KT-10	Wilms	0.358	1.2	>4	0.84	0.258	PD1
KT-11	Wilms	0.111	1.1	>4	0.69	0.093	PD1
KT-13	Wilms	0.713	0.9	>4	1.02	0.786	PD1
SK-NEP-1	Ewing	0.535	0.9	>4	1.11	0.315	PD1
EW5	Ewing	0.104	1.3	>4	0.79	0.315	PD1
EW8	Ewing	0.349	0.9	>4	0.86	0.247	PD1
TC-71	Ewing	0.066*	0.8	>4	1.54	0.123*	PD1
CHLA258	Ewing	0.381	1	>4	0.9	0.786	PD1
Rh28	ALV RMS	0.902	1	>4	1.29	0.684	PD1
Rh30	ALV RMS	0.577	1	>4	1	0.829	PD1
Rh30R	ALV RMS	0.72	0.9	>4	1	0.739	PD1
Rh41	ALV RMS	0.6	1.6	>4	0.62	0.075	PD2
Rh18	EMB RMS	0.033*	0.5	>4	1.82	0.029*	PD1
BT-28	Medulloblastoma	0.001*	1.1	>4	0.72	0.035*	PD1
BT-45	Medulloblastoma	0.125	0.9	>4	1.13	0.165	PD1
BT-46	Medulloblastoma	0.992	1	>4	1.02	1.000	PD1
BT-50	Medulloblastoma	0.042*	0.8	>4	1.06	0.436*	PD1
GBM2	Glioblastoma	0.705	1	>4	0.97	0.684	PD1
BT-39	Glioblastoma	0.093	1.1	>4	0.96	0.218	PD1
D645	Glioblastoma	0.302	1.1	>4	0.96	0.661	PD1
D456	Glioblastoma	0.257	0.9	>4	1.03	0.604	PD1
NB-SD	Neuroblastoma	0.445	1	>4	0.96	1.000	PD1
NB-1771	Neuroblastoma	0.083	1.1	>4	0.77	0.182	PD1
NB-1691	Neuroblastoma	0.769	1.1	>4	1.11	0.912	PD1
NB-EBc1	Neuroblastoma	0.304	1	>4	0.89	0.579	PD1
CHLA-79	Neuroblastoma	0.251	1.1	>4	0.91	0.605	PD1
NB-1643	Neuroblastoma	0.067	1.2	>4	0.76	0.165	PD1
OS-1	Osteosarcoma	0.655	0.9	>4	0.82	0.340	PD1
OS-2	Osteosarcoma	0.224	1.1	>4	0.83	0.278	PD1
OS-17	Osteosarcoma	0.297	1	>4	0.82	0.173	PD1
OS-33	Osteosarcoma	0.974	0.9	>4	1.02	0.063	PD1
OS-31	Osteosarcoma	0.749	0.9	>4	0.83	0.133	PD1
ALL-3	ALL B-precursor	0.132	1.1	>25	.	.	PD1
ALL-4	ALL B-precursor	0.598	1	>25	.	.	PD1
ALL-7	ALL B-precursor	0.274	1	>25	.	.	PD1
ALL-8	ALL T-cell	0.883	0.9	>25	.	.	PD1
ALL-16	ALL T-cell	0.399	0.9	>25	.	.	PD1
ALL-17	ALL B-precursor	1	1	>25	.	.	PD1

- * indicates lines in which the KM days to event were greater in the controls than the treated.
- Tumor Volume T/C: Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days).
- 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.

EGFR and ErbB2 Expression

- **EGFR and ErbB2 expression were evaluated using Affymetrix U133 Plus 2.0 arrays. With the exception of the ALL xenografts and cell lines, most of the PPTP's preclinical models showed evidence of either EGFR or ErbB2 expression.**
- **For the PPTP xenografts, ErbB2 was most consistently expressed in the rhabdoid tumor, Wilms tumor, and ependymoma panels. Most xenografts in the osteosarcoma panel and Ewing sarcoma also demonstrated ErbB2 expression. ErbB2 expression was low or absent in most of the xenografts of the neuroblastoma panels and the ALL panel.**



CONCLUSIONS

- **The response of the PPTP cell lines to lapatinib corresponds to the pattern of *in vitro* response previously described for adult cancer cell lines that do not overexpress either EGFR or ErbB2. There were no PPTP cell lines with IC_{50} values less than 0.25 mM that typify the response to lapatinib for EGFR- or ErbB2-driven adult cancer cell lines.**
- **Therefore, the *in vitro* growth inhibitory effects observed for lapatinib against the PPTP's cell lines likely reflect non-specific tyrosine kinase inhibition.**
- **Lapatinib demonstrated little or no *in vivo* activity against the PPTP's solid tumor or leukemia xenografts.**
- **The lack of significant *in vivo* activity for lapatinib mirrors the lack of activity observed for the EGFR inhibitor gefitinib against 10 pediatric xenografts, including neuroblastoma, rhabdomyosarcoma, osteosarcoma, and glioblastoma xenografts (Stewart, et al. Cancer research 2004:64:7491-7499).**
- **The absence of significant lapatinib *in vivo* activity despite EGFR and/or ErbB2 expression by many of the PPTP's xenografts is similar to the lack of activity for imatinib in the pediatric solid tumor setting despite KIT and/or PDGFR expression by many pediatric cancers.**
- **Unlike the situation for breast cancer in which high-level ErbB2 expression occurs in a large subset of patients as a result of ErbB2 gene amplification, ErbB2 gene amplification is uncommon for pediatric cancers. Similarly, no pediatric tumors are known to have significant rates of EGFR activating mutations.**
- **Additional studies are required to fully understand the biological basis for the lack of responsiveness of pediatric cancer cell lines and xenografts to lapatinib and other ERB family inhibitors. However, it appears that in contrast to the adult cancer setting, the role of agents targeted against ERB family members may be quite limited in the pediatric setting.**

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