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Initial Testing (Stage 1) of Lapatinib by the Pediatric Preclinical Testing Program

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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.

Methods: Lapatinib was tested against the cell lines of the PPTP *in vitro* panel at concentrations ranging from 1.0 nM to 10.0 μ M. Lapatinib was tested against the xenografts of 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). Lapatinib pharmacokinetic parameters were determined in *scid*^{-/-} mice.

Results: The median IC₅₀ value for lapatinib against the entire PPTP cell line panel was 6.84 μ M (range, 2.08 μ M to > 10.0 μ M). 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 xenografts tested. No objective responses were observed in any of the solid tumor panels or in the ALL panel. Lapatinib systemic exposure was consistent with previously observed values.

Conclusions: Lapatinib has little activity against the xenografts of the PPTP's *in vivo* panel, and its *in vitro* activity occurs at concentrations above those associated with specific EGFR/ErbB2 inhibition. These results likely reflect lack of ErbB2 overexpression in the models studied and suggest that adult and pediatric cancers may fundamentally differ in the applicability of EGFR family members as therapeutic targets.

Keywords

Preclinical Testing; Developmental Therapeutics; Lapatinib

INTRODUCTION

The epidermal growth factor receptor (EGFR) family includes four members: EGFR (HER1), human epidermal growth factor receptor 2 (ErbB2, HER2), HER3 (ErbB3), and HER4 (ErbB4) [1]. A range of activating EGFR mutations have been reported in high grade gliomas and non-small cell lung cancer [2]. ErbB2 plays an oncogenic role in breast cancer through protein overexpression which in this malignancy is related to gene amplification [3]. The importance of EGFR family signaling in these and other adult cancers has led to large drug development programs that have culminated in licensing of several monoclonal antibodies and small molecule inhibitors targeted to EGFR and ErbB2 for a variety of indications. Lapatinib (GW572106), which recently achieved a registered indication for refractory breast cancer with ErbB2 overexpression, is a small molecule reversible tyrosine kinase inhibitor with IC₅₀ values of approximately 10 nM against EGFR and ErbB2 in cell-free biochemical kinase assays [4]. Lapatinib induced growth arrest and/or apoptosis in EGFR- and ErbB2-dependent tumor cell lines [5,6], and it induced marked *in vivo* growth inhibition in EGFR- and ErbB2-overexpressing xenografts [4,7].

The NCI-supported Pediatric Preclinical Testing Program (PPTP) is a comprehensive program to systematically evaluate new agents against a set of molecularly characterized childhood solid tumor and leukemia models. As the role of EGFR- and ErbB2-targeted agents for pediatric cancers is not well defined, lapatinib was selected for evaluation against the PPTP's models to help establish priorities for additional preclinical testing and for clinical evaluations of lapatinib against pediatric cancers.

MATERIALS AND METHODS

In vitro testing

In vitro testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viability [8]. Cells were incubated in the presence of lapatinib for 96 hours at concentrations from 1 nM to 10 μ M and analyzed as previously described [9].

In vivo tumor growth inhibition studies

CB17SC-M *scid*^{-/-} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [10-12]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [13]. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Tumor volumes (cm³) [solid tumor xenografts] or percentages of human CD45-positive [hCD45] cells [ALL xenografts] were determined as previously described [14]. Responses were determined using three activity measures as previously described [14]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions.

Statistical methods

The exact log-rank test, as implemented using Proc StatXact for SAS[®], was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies.

Drugs and Formulation

Lapatinib was provided to the PPTP by GlaxoSmithKline through the Cancer Therapy Evaluation Program (NCI). Lapatinib was dissolved in a vehicle of 0.5% (w/w) hydroxypropylmethylcellulose / 0.1% Tween 80 in water and administered by oral gavage, twice-daily X 5 for 6 consecutive weeks at a dose of 160 mg/kg. Lapatinib was provided to each consortium investigator in coded vials for blinded testing.

Pharmacokinetic testing

Lapatinib was administered at 160 mg/kg as described above and plasma samples were collected by cardiac stick with heparinized syringes at 0.5, 1, 2, 4, 6, 8, and 12 hours following administration. Plasma was stored at -80C until analysis. Samples (100 μ L) were prepared using solid phase extraction (SPE) columns, and 6.0 μ L of the re-constituted eluate was injected onto a Phenomenex[®] CuroSil-PFP 3 μ m analytical column (50mm $\times$ 2.0 mm) with an isocratic mobile phase consisting of acetonitrile/5.0mM ammonium formate (pH 3.0)/formic acid (v/v/v = 1000:50:1). Analytes were detected with a PE SCIEX API-3000 LC-MS/MS system at unit (Q1) and low (Q3) resolution in positive multiple-reaction monitoring mode (m/z 581 (precursor ion) to m/z 364 (product ion) for lapatinib). The mean recovery for lapatinib was 75%, with a lower limit of quantification of 15 ng/mL (S/N = 11.3, CV $\leq$ 14%). This method was validated over a linear range of 100–10,000 ng/mL, using deuterated lapatinib (D6-lapatinib) as the internal standard.

Lapatinib concentration-time data were modeled by maximum likelihood in ADAPT II [15]. A one-compartment pharmacokinetic model with first-order elimination was fit to the lapatinib plasma concentration-time data. The pharmacokinetic parameters estimated with the model included apparent oral clearance (CL/F), apparent volume of distribution (Vc/F), absorption rate constants (ka). The area under the concentration-time curve to 12 hours (AUC_{0 $\rightarrow$ 12}) was calculated from the simulated data obtained from the estimates of the pharmacokinetic parameters using the log-linear trapezoidal method.

Western Blotting

Western blot analyses were performed as previously described with minor modifications [16]. Primary antibodies to ErbB2, p-ErbB2, and GAPDH (Cell Signaling) were used.

RESULTS

Lapatinib in vitro testing

Lapatinib variably inhibited growth of the cell lines from the PPTP *in vitro* panel (Table I). Fifteen of 23 cell lines achieved at least 50% growth inhibition. The median IC₅₀ for the entire panel was 7.76 μ M with a range of 4.23 μ M to >10 μ M.

Lapatinib in vivo testing

Lapatinib was evaluated in 41 xenograft models. Seven of 787 mice died during the study (0.9%), with 1 of 385 in the control arms (0.3%) and 6 of 402 in the lapatinib treatment arms (1.5%). A complete summary of results is provided in Supplemental Table I, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice

with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values. Lapatinib induced significant differences in EFS distribution compared to controls in 1 of 41 xenografts tested (Table II). No xenografts met the criteria for intermediate activity for the PPTP EFS T/C 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).

Lapatinib pharmacokinetic testing

A one-compartment model fit the lapatinib concentration-time data well (Figure 1). The pharmacokinetic parameters obtained were: $k_e = 0.35 \text{ hr}^{-1}$, $V_d = 8.1 \text{ L/kg}$, $k_a = 1.74 \text{ h}^{-1}$, apparent oral clearance = 2.84 L/h/kg , and half-life = 1.98 hr . The $\text{AUC}_{0 \rightarrow 12\text{hr}}$ was $55.4 \text{ } \mu\text{g/mL} \cdot \text{hr}$.

Expression of ErbB receptors

The relative expression of ErbB2 in the PPTP *in vivo* models is shown in Figure 2. Expression was relatively high in the kidney tumor panel, Ewing family tumor and non-glioblastoma brain tumor models. Protein levels for ErbB2 and p-ErbB2 were determined in selective xenograft models including Ewing sarcoma (EW5, EW8, SK-NEP-1), rhabdomyosarcoma (Rh30, Rh36, Rh41) and two brain tumors, BT-28 (medulloblastoma) and BT-44 (ependymoma). Consistent with the Affymetrix data, ErbB2 was detected in Ewing sarcoma and non-glioblastoma brain tumors, but not rhabdomyosarcomas. However, compared to the level of expression of ErbB2 in UACC-812 cells that have amplified ErbB2, protein levels of ErbB2 in pediatric xenograft models were much lower. Further, activated ErbB2 (phosphorylation of Y1221/1222) was not detected in any of the pediatric tumor models (Figure 3).

DISCUSSION

Although the activity of lapatinib and other inhibitors of ErbB signaling is established for several adult malignancies, at this time there is a very limited body of clinical experience for EGFR inhibitors in children with cancer. Hence it was of interest to determine the antitumor activity of lapatinib against the PPTP's *in vitro* and *in vivo* panels of pediatric tumors. The IC_{50} values for the PPTP cell lines were comparable to those of adult cancer cell lines that do not overexpress either EGFR or ErbB2 [4,5]. The PPTP cell lines showed growth inhibitory effects only at high concentrations that likely reflect non-specific tyrosine kinase inhibition. The *in vivo* testing results are consistent with the *in vitro* testing results in showing no apparent dependence upon EGFR or ErbB2 signaling for the xenografts in the PPTP's *in vivo* solid tumor and ALL panels. This lack of activity is unlikely to be the result of inadequate dosing or scheduling based on prior reports. Pharmacokinetic studies for lapatinib were conducted to assess the disposition of lapatinib in the *scid*^{-/-} mouse model, primarily to assure that lapatinib was absorbed in the formulation administered. As shown in the results, lapatinib was rapidly absorbed with maximum concentrations reached around two hours after oral administration. The volume of distribution, clearance, and half-life values reported in this study are consistent with those reported in previous studies conducted in other mouse models. Although some variability has been reported in lapatinib oral absorption in the mouse model, the systemic exposure at the 160 mg/kg dosage administered for this pharmacokinetic study is consistent with that observed for children in the Pediatric Brain Tumor Consortium phase I clinical trial of lapatinib at its MTD of 900 mg/m^2 (i.e., median $\text{AUC}_{0 \rightarrow 12} 55.3 \text{ } \mu\text{g/mL} \cdot \text{hr}$) [17].

The lack of activity observed for lapatinib is disappointing in light of published reports suggesting that EGFR and ErbB2 may be therapeutic targets for several childhood cancers. However, these reports primarily described EGFR or ErbB2 expression in pediatric cancers or demonstrated an association of expression with prognosis, rather than demonstrating the activity of therapy directed towards these targets. In previous reports EGFR expression had been described for osteosarcoma [18], Wilms tumor, hepatoblastoma, neuroblastoma [19], synovial sarcoma, pediatric high-grade gliomas [20], and ependymoma [21]. ErbB2 expression is detected in a wide range of childhood cancers [22], including some cases of ALL and a substantial proportion of medulloblastoma and ependymoma cases [21]. Expression of ErbB2 has been associated with poor prognosis in patients with osteosarcoma [23], although not all reports confirm this association [24]. Unlike the situation for breast cancer, ErbB2 gene amplification is uncommon in osteosarcoma, medulloblastoma, and ependymoma. The previous reports of EGFR and ErbB2 expression in pediatric cancers are generally concordant with the gene expression data for the PPTP's xenografts, with either EGFR or ErbB2 gene expression noted for most xenografts among the solid tumor panels (Figure 2). However, relative to the UACC-812 cell line that harbors amplification of Erb2, expression of ErbB2 at the protein level in pediatric xenografts is low (Figure 3). Further, activated ErbB2 (phosphorylated at Y1221/1222) was not detected in any xenograft model.

In summary, lapatinib has little activity against the xenografts of the PPTP's *in vivo* panel, and its *in vitro* activity occurs at concentrations above those associated with specific EGFR/ ErbB2 inhibition. These results likely reflect lack of ErbB2 overexpression in the models studied and suggest that adult and pediatric cancers may fundamentally differ in the applicability of EGFR family members as therapeutic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Lapatinib pharmacokinetic analysis, a one-compartment model fit of the lapatinib concentration-time data. The pharmacokinetic parameters obtained were: $k_e = 0.35 \text{ hr}^{-1}$, $V_d = 8.1 \text{ L/kg}$, $k_a = 1.74 \text{ h}^{-1}$, apparent oral clearance = 2.84 L/h/kg , and half-life = 1.98 hr . The $AUC_{0 \rightarrow 12\text{hr}}$ was 55.4 µg/mL*hr .

Figure 2.

Gene expression for ErbB2 in xenograft tissues obtained from Affymetrix HG-U133 Plus 2.0 as visualized using GeneSifter software (VizX Labs, Seattle, WA). Gray indicates an absent call from Affymetrix quality control.

Figure 3. ErbB2 and phospho-ErbB2 expression in selected pediatric xenografts: Ewing family tumors (EW5, EW8, and SK-NEP-1); medulloblastoma (BT-28); ependymoma (BT-44), and rhabdomyosarcoma (Rh30, Rh36, and Rh41).

Table 1Activity of Lapatinib against Cell Lines in the PPTP *in Vitro* Panel

Cell Line	Status	Histology	Maximal Inhibition (%)	EC ₅₀ (nM)	IC ₅₀ (nM)
RD		Rhabdomyosarcoma	97	>10	>10
Rh41	Post-Therapy	Rhabdomyosarcoma	96	7.66	7.76
Rh18	Diagnosis	Rhabdomyosarcoma	99	>10	>10
Rh30	Diagnosis	Rhabdomyosarcoma	98	7.60	7.61
BT-12	Diagnosis	Rhabdoid	72	7.58	7.70
CHLA-266	Diagnosis	Rhabdoid	48	>10	>10
TC-71	Post-Therapy	Ewing	79	4.06	4.39
CHLA-9	Diagnosis	Ewing	94	4.22	4.23
CHLA-10	Post-Therapy	Ewing	100	4.76	4.98
CHLA-258	Post-Bone Marrow Transplant	Ewing	83	>10	>10
SJ-GBM2	Post-Therapy	Glioblastoma	87	5.02	6.06
NB-1643	Diagnosis	Neuroblastoma	88	>10	>10
NB-EBc1	Post-Therapy	Neuroblastoma	95	>10	>10
CHLA-90	Post-Bone Marrow Transplant	Neuroblastoma	87	>10	>10
CHLA-136	Post-Bone Marrow Transplant	Neuroblastoma	87	>10	>10
COG-LL-317	Post-Therapy	ALL T-cell	95	5.09	5.72
NALM-6	Post-Therapy	ALL B-precursor	95	5.69	7.21
RS4;11¹	Post-Therapy	ALL B-precursor	87	6.71	8.64
MOLT-4	Post-Therapy	ALL T-cell	88	2.68	5.28
CCRF-CEM		ALL T-cell	90	6.20	7.71
Kasumi-1	Post-Bone Marrow Transplant	AML	83	5.68	8.76
Karpas-299	Post-Therapy	ALCL	87	8.09	9.30

Cell Line	Status	Histology	Maximal Inhibition (%)	EC ₅₀ (μM)	IC ₅₀ (μM)
Ramos-RA1		NHL	88	1.90	6.28
Median			88	6.99	7.76
Minimum			48	1.90	4.23
Maximum			100	>10	>10

Table II

Activity for Laptinib against the PPTP *in Vivo* Panel

Line Description	Tumor Type	KM Estimate of Median Time to Event	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	T/C Activity	EFS Activity	Response Activity
BT-29	Rhabdoid	12.2	0.241	1.3	>4	0.62	0.029	Low	Low	Low
KT-16	Rhabdoid	15.3	0.93	1	>4	0.98	0.912	Low	Low	Low
KT-14	Rhabdoid	17.5	0.494	1.1	>4	0.97	0.211	Low	Low	Low
KT-10	Wilms	8	0.358	1.2	>4	0.84	0.258	Low	Low	Low
KT-11	Wilms	15.6	0.111	1.1	>4	0.69	0.093	Low	Low	Low
KT-13	Wilms	5.3	0.713	0.9	>4	1.02	0.796	Low	Low	Low
SK-NEP-1	Ewing	6.3	0.535	0.9	>4	1.11	0.315	Low	Low	Low
EW5	Ewing	18.6	0.104	1.3	>4	0.79	0.315	Low	Low	Low
EW8	Ewing	9.1	0.349	0.9	>4	0.86	0.247	Low	Low	Low
TC-71	Ewing	5.4	0.066*	0.8	>4	1.54	0.123	Low	Low	Low
CHLA258	Ewing	15.4	0.381	1	>4	0.9	0.750	Low	Low	Low
Rh28	ALV RMS	14.4	0.902	1	>4	1.29	0.684	Low	Low	Low
Rh30	ALV RMS	17	0.577	1	>4	1	0.829	Low	Low	Low
Rh30R	ALV RMS	14.3	0.72	0.9	>4	1	0.739	Low	Low	Low
Rh41	ALV RMS	11	0.6	1.6	>4	0.62	0.075	Low	Low	Int
Rh18	EMB RMS	11	0.033*	0.5	>4	1.82	0.029	Low	Low	Low
BT-28	Medulloblastoma	4.4	0.031	1.1	>4	0.72	0.035	Low	Low	Low
BT-45	Medulloblastoma	10.9	0.125	0.9	>4	1.13	0.165	Low	Low	Low
BT-46	Medulloblastoma	10.8	0.992	1	>4	1.02	1.000	Low	Low	Low
BT-50	Medulloblastoma	15.6	0.042*	0.8	>4	1.06	0.436	Low	Low	Low
GBM2	Glioblastoma	9.9	0.705	1	>4	0.97	0.684	Low	Low	Low
BT-39	Glioblastoma	16.6	0.093	1.1	>4	0.96	0.218	Low	Low	Low
D645	Glioblastoma	8.1	0.302	1.1	>4	0.96	0.661	Low	Low	Low
D456	Glioblastoma	6.1	0.257	0.9	>4	1.03	0.604	Low	Low	Low

Line Description	Tumor Type	KM Estimate of Median Time to Event	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	T/C Activity	EFS Activity	Response Activity
NB-SD	Neuroblastoma	15.8	0.445	1	>4	0.96	1.000	Low	Low	Low
NB-1771	Neuroblastoma	9.6	0.083	1.1	>4	0.77	0.182	Low	Low	Low
NB-1691	Neuroblastoma	5.9	0.769	1.1	>4	1.11	0.912	Low	Low	Low
NB-EBc1	Neuroblastoma	12.4	0.304	1	>4	0.89	0.579	Low	Low	Low
CHLA-79	Neuroblastoma	20.3	0.251	1.1	>4	0.91	0.605	Low	Low	Low
NB-1643	Neuroblastoma	10.2	0.067	1.2	>4	0.76	0.165	Low	Low	Low
OS-1	Osteosarcoma	19.9	0.655	0.9	>4	0.82	0.340	Low	Low	Low
OS-2	Osteosarcoma	16	0.224	1.1	>4	0.83	0.278	Low	Low	Low
OS-17	Osteosarcoma	17.5	0.297	1	>4	0.82	0.173	Low	Low	Low
OS-33	Osteosarcoma	9.4	0.974	0.9	>4	1.02	0.063	Low	Low	Low
OS-31	Osteosarcoma	19.7	0.749	0.9	>4	0.83	0.133	Low	Low	Low
ALL-3	ALL B-precursor	11.1	0.132	1.1	>25	.			Low	Low
ALL-4	ALL B-precursor	1.8	0.598	1	>25	.			Low	Low
ALL-7	ALL B-precursor	2	0.274	1	>25	.			Low	Low
ALL-8	ALL T-cell	5.3	0.883	0.9	>25	.			Low	Low
ALL-16	ALL T-cell	10.3	0.399	0.9	>25	.			Low	Low
ALL-17	ALL B-precursor	2	1	1	>25	.			Low	Low

* KM days greater in Control than Treated.