**Abstract**

Pentaxin II in vitro Testing Methods

Methods: In vitro testing was performed using EGFR/Erbb2-expressing xenograft lines of cell lines and clinical samples. The cell lines included BT-20, BT-45, Rh18, and Rh30, which were derived from human breast tumors. The xenografts included BT-29, CHLA-9, CHLA-4, and CHLA-136, which were derived from human neuroblastoma tumors. The cell lines and xenografts were grown and maintained in accordance with standard laboratory procedures. The cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The xenografts were maintained in Matrigel-coated tissue culture dishes and grown in a humidified incubator at 37°C with 5% CO2.

1. **Lapatinib in Vitro Activity**

- The PPTP cell lines had similar patterns of response to lapatinib, with activity observed almost exclusively at concentrations ≥ 100 nM. Some lines showed increased activity at concentrations > 1000 nM.
- Examples of dose response curves illustrating the general pattern of responses are shown below for the Ewing sarcoma cell line CHLA-9 (left) and CHLA-10 (right).

2. **Lapatinib in Vivo Activity**

Stage 1 testing involves testing on an agent against the entire PPTP panel. Treatment was initiated at a dose of 160 mg/kg twice daily (2 days on, 2 days off) for 6 weeks. Tumor growth inhibition (TGI) was defined as the percentage of treated animals with a tumor volume ≤ 60% of the initial volume.

3. **Methods for PPTP in Vivo Testing**

- Lapatinib was well tolerated in vivo using a twice-daily oral administration schedule for 6 weeks (2 days on, 2 days off). Testing was performed using 10 untreated animals per xenograft line.
- Tumors were measured at least weekly using calipers and tumor volume was calculated using the formula (length x width x height)/2. The median tumor volume at the end of treatment was used to calculate the T/C ratio. The median T/C ratio for each xenograft line was compared to the control T/C ratio using the Wilcoxon rank-sum test.
- The activity observed for the EGFR inhibitor lapatinib was assessed using the following criteria:
  - A statistically significant difference in EFS distribution compared to control (p-value < 0.01).
  - A median T/C ratio of < 0.5 for EFS.
  - A median T/C ratio of < 0.75 for tumor volume.

4. **EGFR and Erbb2 Expression**

- The response of the PPTP cell lines to lapatinib corresponds to the pattern of in vitro response previously described for adult cancer cell lines. The median IC50 value for lapatinib was calculated for each xenograft line. The median EFS was determined for each xenograft line. The median T/C ratio was determined for each xenograft line. The median T/C ratio was compared to the control T/C ratio using the Wilcoxon rank-sum test.

** 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. The median IC50 value for lapatinib was calculated for each xenograft line. The median EFS was determined for each xenograft line. The median T/C ratio was determined for each xenograft line. The median T/C ratio was compared to the control T/C ratio using the Wilcoxon rank-sum test.

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