Abstract #114

Anti-tumor activity of tarloxotinib, a hypoxia-activated EGFR/HER2 TKI, in HER2 driven cell lines
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Introduction

- NSCLC is the leading cause of cancer-related deaths; 
  - but strategies using oncogene-targeted therapies have led to improvements in clinical outcomes for subsets of patients.
- Aberrant HER2 expression (amplifications, mutations or overexpression) in NSCLC and other cancers can cause constitutive activation and downstream signaling. HER2 gene amplification is present in 2-4% of NSCLC, while HER2 activating mutations are present in 3% of NSCLC.
- Other oncogenic alterations such as ERBB gene fusions and EGFR/HER2 domain duplications may be amenable to HER2/EGFR kinase inhibitors.1,2

- HER2 alterations in lung cancer showed resistance to known TKIs, thus, new therapies need to be developed for lung cancer patients with HER2 aberrations.3
- Solid tumors, including lung cancer, harbor of "hypoxia" which can create a hostile microenvironment with low pH, chemotherapy, and immunotherapy. To transform this poor angiogenesis feature into a vulnerability, tarloxotinib was designed as a prodrug that is activated under hypoxic conditions to release the hypoxic EGFR/HER2 inhibitor (tarloxotinib).3,4

Tarloxotinib can increase the therapeutic ratio over conventional anti-HER2/HER2 therapies by inhibiting EGF signaling.

- In this study, we evaluate the effect of tarloxotinib on cell lines harboring HER2 alterations, EGFR kinase domain duplication and a D0AC-NRG1 fusion.

Figure 1. A) Schematic of hypoxic regions in tumor tissue indicating the expression of VEGF in infiltrating cells. B) A box model illustrating the notion of a hypoxic trigger (blue) to tarloxotinib significantly reduces the polarity of the probe, allowing for administration at higher relative concentrations than the cognate TKI (tarloxotinib).

Figure 2. Tarloxotinib-E inhibits ErbB family members phosphorylation and signaling in HER2 and HER2-driven cell lines. Cells were treated with the indicated doses of ErbB1, ErbB2, ErbB3, or ErbB4-EGFR (active drug) for 2 hours, lysed and analyzed by Western. Western blots show ErbB family members phosphorylated: pERBB1 (Y1173), pAKT (S473), p53R (Y202/204), pHER2 (Y1221/1222), pERBB3 (Y1068).

Figure 3. Tarloxotinib-E inhibits proliferation of cell lines harboring HER2 alterations. A) Dose response curves of cell viability of HER2 and HER2 with gain of kinase domain in hypoxic tumors. Calu-3 (HER2 amp), MDA-MB-175 (HER2 gene amplification) and A172 (Glia) (HER2 gene compound duplication). Cells were treated with trastuzumab, Gefitinib, Tarloxotinib (pro-drug) and Tarloxotinib and Gefitinib (active-drug) for 72 hours and measured by MTS. Experiments were done in triplicate, mean ± SEM is plotted. B) Table summarizing IC50 values of the proliferation experiments.

Figure 4. Tarloxotinib-E induces apoptosis in HER2 altered and NRG1 fusion cell lines. IntraCyte quantification of H1781, Calu-3 and MDA-MB-175 (HER2) cells treated with 10,000nM of Tarloxotinib-E, gefitinib, afatinib or control. Apoptotic cells are labeled with caspase-3/7 green reagent and quantified in real time using the IncuCyte live-cell analysis system.

Figure 5. Tarloxotinib-E inhibits interaction of HER2 with subunit p50 of PKD. Proximity ligation assay was performed in H1781 cells treated with tarloxotinib-E for 30 time. Rac1 represents a positive interaction of HER2 with p50 or GRB2.

Conclusions

- Tarloxotinib-E, in vitro, may be amenable in HER2 dependent cell lines and tarloxotinib-E has 80-100 fold greater activity compared to tarloxotinib in vivo under normoxic conditions.
- Tarloxotinib-E inhibits HER2 and HER2 phosphorylation and downstream signaling in vitro. Additionally, tarloxotinib-E inhibits phosphorylation of EGFR and HER2 kinase domain duplication cell line.
- HER2 altered cell lines favor PKD/akt signaling over erbb2, signaling.
- The active-drug induces apoptosis after 12-48 hours of exposure.
- Tarloxotinib significantly reduces tumor volume and proliferation in two HER2 xenograft models.

References

Funding: Developmental Therapeutics Program, University of Colorado Cancer Center (NINCI P30CA046593)