Resistance to tucatinib associated with hyperactive EGFR could be overcome by co-targeting HER2 and EGFR in HER2-positive breast cancer models

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Background

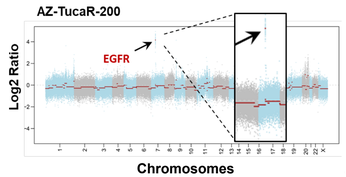
Tucatinib (Tuca) is a potent HER2-selective small molecule tyrosine kinase inhibitor (TKI) approved for use in HER2+ metastatic breast cancer (MBC), including in patients with brain metastasis. In current practice, despite the expanding use of other anti-HER2 agents like TDXd in the 2nd and potentially the 1st line setting, Tuca continues to be a key treatment choice for HER2+ MBC, mostly after TDxd. We previously showed that EGFR amplification leading to HER pathway reactivation is associated with acquired resistance to Tuca in HER2+ BC models, which could be overcome using dual/pan-HER TKIs or the combination of potent EGFR and HER2 inhibitors (Liao et al, Can Res, 2022). Building on these key prior in vitro findings, here we sought to validate the efficacy of this treatment strategy in the in vivo setting.

Experimental Models and Approach

- **Models**: HER2+ BT474/AZ acquired Tuca resistant derivatives (TucaR) generated through long-term exposure to gradually increasing doses of Tuca
- **Prior reported findings:**
  - Resistance to tucatinib is associated with acquisition of EGFR amplification and reactivation of the HER pathway *(SABCS 2021)*
  - TucaR derivatives possess enhanced migratory capabilities than naïve parental cells, which could be effectively inhibited by the EGFR-specific TKI gefitinib, either alone or in combination with Tuca
  - Tucatinib resistance associated with EGFR amplification could be overcome using EGFR specific inhibitors or by a more complete blockade of the HER receptor layer
- ***In vivo* xenograft studies:**
  - Develop xenografts by s.c. injection of GFP/Luc-tagged TucaR cells
  - *Treatments*: EGFR specific inhibitors : 1) the TKI Gefitinib (Gef), or 2) the monoclonal antibody Cetuximab (Cet), either alone or together with Tuca
  - *Major Endpoints*: Monitor tumor growth (using caliper and IVIS imaging) and metastasis (IVIS imaging)

Results

I. BT474 cells acquire EGFR amplification at time of resistance to tucatinib

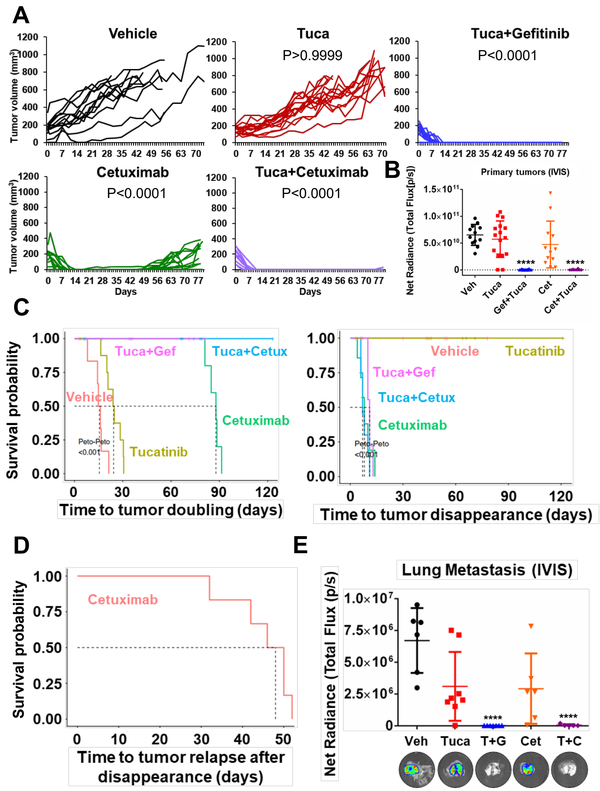


Whole exome sequencing analysis of BT474/AZ-P and their cognate acquired TucaR derivatives revealed amplification of EGFR in the TucaR model.

Jamunarani Veeraraghavan, SABCS 2021

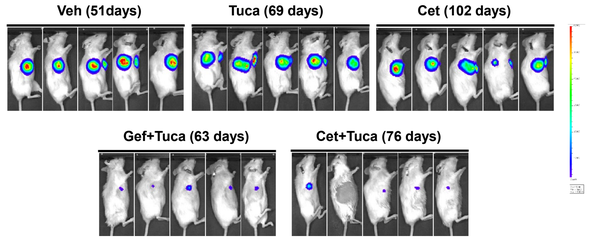
Results

II. Simultaneous blockade of HER2 and EGFR overcomes tucatinib resistance associated with high EGFR



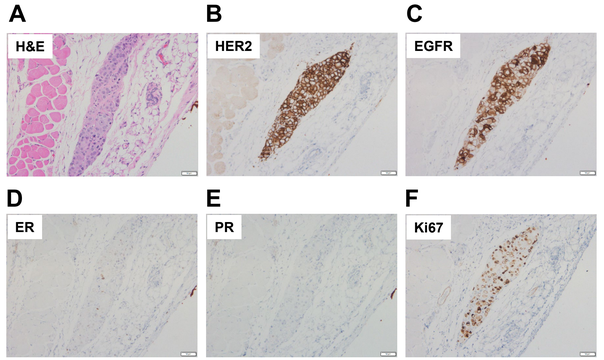
(A) Growth of GFP/Luc-tagged TucaR xenografts treated with vehicle, Tuca (100mg/kg, oral, daily), Gefitinib (Gef; 100mg/kg, oral, 5 days/week) or Cetuximab (Cet; 30mg/kg, i.p., twice weekly) alone or in combination. Statistical analysis: one-way ANOVA. **(B)** Scatter plot of photon flux signals from IVIS imaging of primary tumors at study endpoint. Kaplan–Meier curves showing **(C)** time to tumor progression (doubling) and regression (halving), and **(D)** time to tumor relapse following complete regression in cetuximab-treated mice. **(E)** Scatter plot of *ex vivo* photon flux from IVIS imaging of lungs at study endpoint. **** P<0.0001

III. Bioluminescence imaging on harvest day revealed minimal/microscopic residual disease in the combination treatment arms



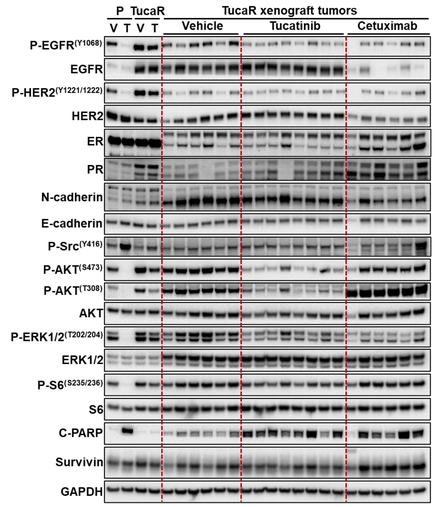
Bioluminescence images captured at the end of the experiment show IVIS signals in mice bearing GFP/Luc-labeled BT474 TucaR tumors treated with Veh, Tuca, Cet, Tuca+Gef, or Tuca+Cet show residual disease at the primary tumor site in the Tuca+Gef and Tuca+Cet arms. Average number of days from start of treatment to tumor harvest for each arm is noted in ().

IV. Residual tumor cluster beneath the skin layer and muscle at the subcutaneous primary tumor site



Representative **(A)** H&E and **(B)** HER2, **(C)** EGFR, **(D)** ER, **(E)** PR, and **(F)** Ki67 IHC images show one of the microscopic residual tumor clusters beneath the skin/muscle layer in a TucaR tumor-bearing mouse with no measurable tumor after Tuca+Gef treatment. Preliminary histopathologic analysis reveal high homogenous HER2 but relatively heterogeneous EGFR and Ki67 levels with very low ER and PR levels (day 35). Images captured at 20× magnification. Scale bar: 50μm.

V. Effect of HER1/2 inhibition on key HER receptors and downstream signaling proteins in the EGFR-amplified BT474/TucaR xenografts



Western blot analysis of key total/phosphorylated (p) proteins in the HER signaling pathway in BT474/AZ parental (P) and TucaR cells treated with vehicle (V) or tucatinib (T), and in TucaR xenografts treated with vehicle, tucatinib, or cetuximab.

Conclusions, Clinical relevance, and Future directions

- Dual HER2/EGFR blockade overcomes tucatinib resistance associated with high EGFR via amplification.
- EGFR monotherapy induced rapid tumor regression in mice, but all tumors eventually recurred on treatment.
- Complete eradication of the primary tumors without recurrence on treatment, as well as elimination of the associated lung metastasis was achieved only with co-targeting HER2 and EGFR.
- Despite complete macroscopic regression, minimal residual tumor cells persisted at the injection site, underscoring the value of imaging-based endpoints and the need for additional targeted agents, including against the estrogen receptor and the cell cycle pathways.
- Our findings emphasize the need for concurrent HER2 and EGFR inhibition to treat HER2+ tumors with high EGFR, a promising therapeutic strategy that warrants further evaluation in clinical trials.

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