Preclinical Characterization of Tucatinib in HER2-Amplified Xenograft and CNS Implanted Tumors

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Background

Tucatinib is an oral, small molecule tyrosine kinase inhibitor for the treatment of HER2+ metastatic breast cancers.

Tucatinib was recently approved for use by the U.S. Food and Drug Administration in combination with trastuzumab and capecitabine for adult patients with advanced unresectable or metastatic HER2-positive breast cancer, including patients with brain metastases based on the results of HER2CLIMB (NCT02614794), a randomized, double-blind, placebo-controlled trial.

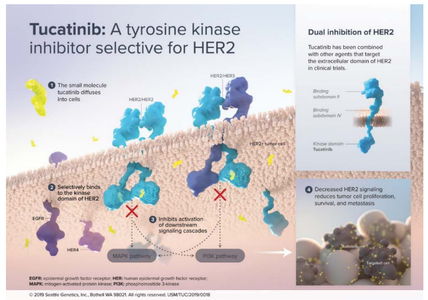
In patients with brain metastases, the addition of tucatinib reduced the risk of cancer progression (brain and/or body) or death (PFS) by 52% compared to trastuzumab and capecitabine alone. *[Murthy, R.K., et al. NEJM (2019)]*

Based on encouraging results from the phase 1b trial, HER2CLIMB-02 (NCT03975647) a phase 3, randomized, international, double-blind trial is now enrolling patients with previously treated HER2⁺ metastatic breast cancer (MBC) with or without brain metastases. Treatment arms include tucatinib or placebo in combination with T-DM1.

Here, we present preclinical data demonstrating that:

- Tucatinib combines with T-DM1 in HER2⁺ breast cancer models in vitro and in vivo for enhanced anti-tumor activity.
- Tucatinib demonstrates anti-tumor activity in both subcutaneous and stereotaxically implanted central nervous system (CNS) tumor models.
- Mechanistically, tucatinib suppresses signaling downstream of HER2 both in vitro and in vivo to inhibit tumor growth.

Tucatinib Proposed Mechanism of Action



The combination of Tucatinib and T-DM1 can have increased cytotoxic activity in vitro

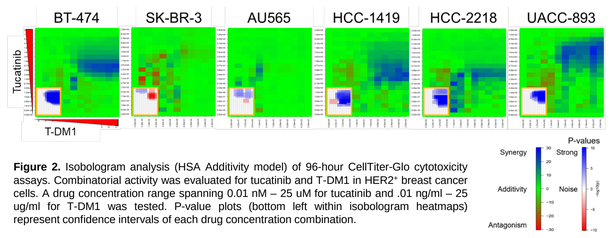


Figure 2. Isobologram analysis (HSA Additivity model) of 96-hour CellTiter-Glo cytotoxicity assays. Combinatorial activity was evaluated for tucatinib and T-DM1 in HER2⁺ breast cancer cells. A drug concentration range spanning 0.01 nM – 25 uM for tucatinib and .01 ng/ml – 25 ug/ml for T-DM1 was tested. P-value plots (bottom left within isobologram heatmaps) represent confidence intervals of each drug concentration combination.

Tucatinib/T-DM1 combinatorial activity is observed in vivo in xenograft models

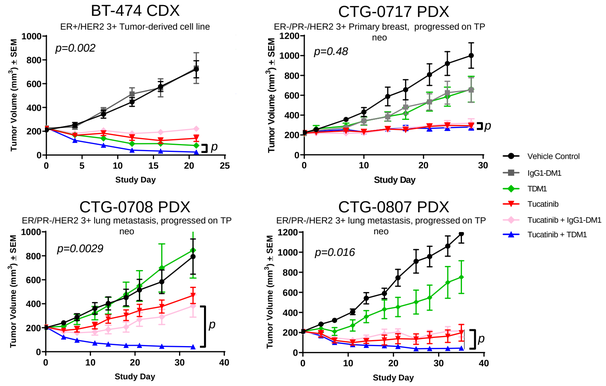


Figure 3. Mean tumor volume over time in HER2⁺ breast cancer BT-474 cell line derived (CDX) and patient-derived (PDX) xenograft models. Tucatinib was administered orally at 50mg/kg bidaily for the duration of the study, while T-DM1 and the IgG-DM1 nonbinding control ADC were dosed at 10 mg/kg (single dose). P-values were determined by t-test analysis comparing tucatinib/T-DM1 combo to the closest single agent arm (ref brackets).

Tucatinib suppresses signaling in HER2⁺ tumors

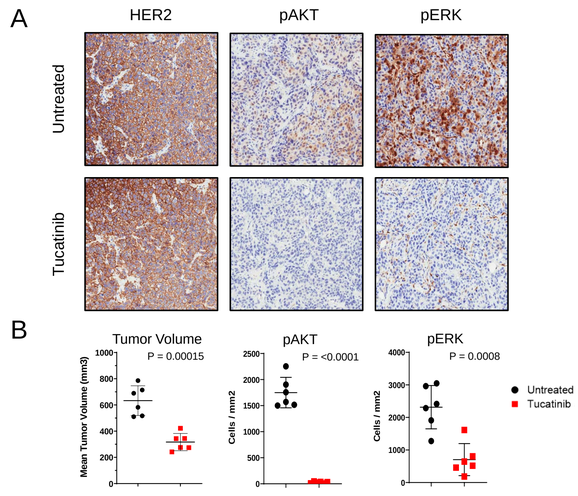


Figure 5. (A) Immunohistochemistry (IHC) staining of BT-474 CDX subcutaneous tumors harvested 7 days after commencement of tucatinib treatment (50 mg/kg bidaily by oral dosing). Tumors were sectioned and stained for HER2, phospho-HER2, phospho-AKT, and phospho-ERK. **(B)** Quantification of tumor volume and weight at time of tumor harvest, along with quantification of IHC signal using Halo® image analysis platform. Unpaired t-test analysis was used to determine p-values.

^{14}C-labeled tucatinib is detectable in CNS tumors at levels higher than normal brain tissue

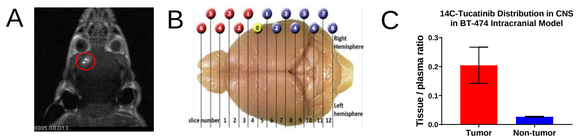


Figure 6. (A) BT-474 tumor cells were injected into the right striatum of nude mice. Thirteen days post-implant, MRI was used to detect tumor volume and location of the tumors. Contrast agent, Gadopentetate dimeglumine was injected intravenously at 0.4 mmol/kg via the caudal vein of mice. The mice were then treated with 70 mg/kg ^{14}C-labeled tucatinib by oral gavage 12 hours apart for a total of 3 doses. Three hours after the third dose the mice were sacrificed, and brains harvested for analysis. **(B)** Serial sections (4mm) were cut in half to bisect the left and right hemispheres, for direct counting of tumor-bearing and non-tumor bearing brain tissue. **(C)** The ^{14}C radioactivity (DPM/g tissue) quantified from tumor and non-tumor bearing brain sections was compared to radioactivity counted in plasma (DPM/mL) to determine the ratio of tissue to plasma distribution.

BT474 cells expressing red-shifted luciferase are engineered to model HER2-amplified CNS tumors

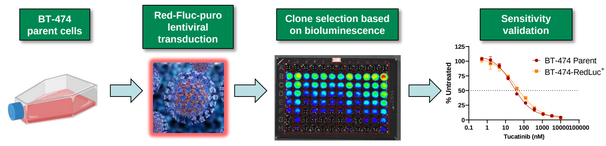


Figure 7. Schematic of development of BT-474-Redluc⁺ cell line. BT-474 parent cells were treated with RediFect™ Red-Fluc-Puro lentiviral particles and put under puromycin selection to create a stable pool. The pool was single cell cloned and clonal cells were evaluated on the IVIS SpectrumCT for relative luminescence per cell. The sensitivity to tucatinib of the top clone was confirmed to be equivalent to that of the parental cell line in a CellTiter-Glo assay.

Intracranial BT474-RedLuc+ Model Development

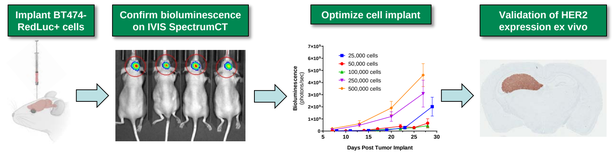


Figure 8. Schematic of in vivo intracranial model development. Stable BT474-RedLuc⁺ clonal cells were stereotaxically implanted into the right striatum of female nude mice bearing 0.18 mg estradiol pellets. Bioluminescence imaging was used to detect the cells in vivo to determine tumor growth rate over time. Implant conditions were evaluated, with the final selection of 250,000 cells providing the optimal dynamic range of luminescence throughout disease progression. Ex vivo HER2 staining by IHC provided validation of CNS tumor location and expression.

Tucatinib demonstrates anti-tumor activity in intracranial CNS tumors

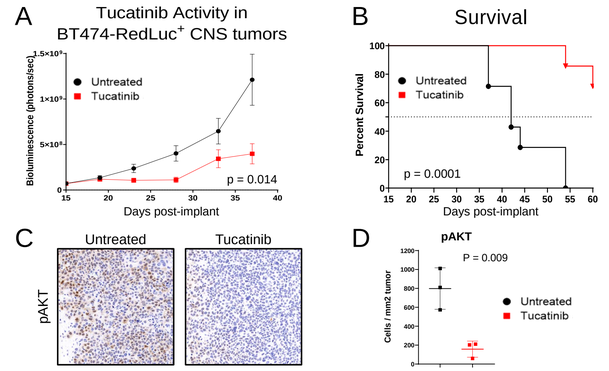
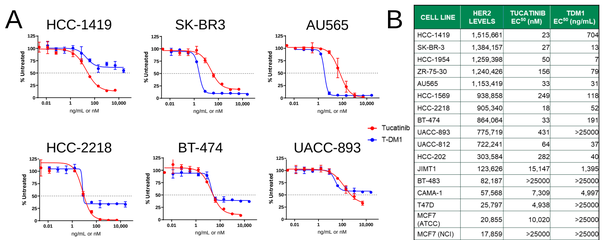


Figure 9. (A) Mean bioluminescence signal over time in BT474-RedLuc⁺ CNS model, as a proxy for tumor growth. Tucatinib was administered bidaily by oral gavage at 75 mg/kg beginning 16 days post-implant, for 21 consecutive days. P-value was determined by Holm-Sidak t-test analysis comparing tucatinib to the untreated control arm at the conclusion of dosing regimen, day 37. **(B)** Kaplan Meier graph showing percent survival following death events where disease-related observations were noted, and mice were humanely euthanized. P-value was calculated using the Log-rank Mantel-Cox test comparing tucatinib-treated mice to untreated. **(C)** IHC staining demonstrating suppression of AKT activation in intracranial tumors after 9 days of tucatinib administration. **(D)** Quantification of phospho-AKT IHC signal utilized the Halo® image analysis platform. Unpaired t-test analysis was used to determine the p-value.

Tucatinib demonstrates potent and selective anti-tumor activity in vitro



B

CELL LINE	HER2 LEVELS	TUCATINIB EC^{50} (nM)	TDM1 EC^{50} (ng/mL)
HCC-1419	1,515,661	23	704
SK-BR-3	1,384,157	27	13
HCC-1954	1,259,398	50	7
ZR-75-30	1,240,426	156	79
AU565	1,153,419	33	31
HCC-1569	938,858	249	118
HCC-2218	905,340	18	52
BT-474	864,064	33	191
UACC-893	775,719	431	>25000
UACC-812	722,241	64	37
HCC-202	303,584	282	40
JIMT1	123,626	15,147	1,395
BT-483	82,187	>25000	>25000
CAMA-1	57,568	7,309	4,997
T47D	25,797	4,938	>25000
MCF7 (ATCC)	20,855	10,020	>25000
MCF7 (NCI)	17,859	>25000	>25000
MDA-MB-231	4,350	16,482	>25000
MDA-MB-436	1,702	17,601	23,878
Hs578T	1,663	12,394	11,493
MDA-MB-468	1	9,617	7,695
DU4475	1	8,973	>25000

Figure 1. (A) Tucatinib and T-DM1 were screened in a panel of breast cancer cell lines spanning a range of HER2 overexpression. Drugs were titrated from 0.01 - 25,000 nM (tucatinib) or 0.01 – 25,000 ng/ml (ADCs) in CellTiter-Glo cytotoxicity assays. **(B)** Summary of EC50 values for tucatinib in CTG assays.

Tucatinib suppresses HER2-mediated signaling in vitro

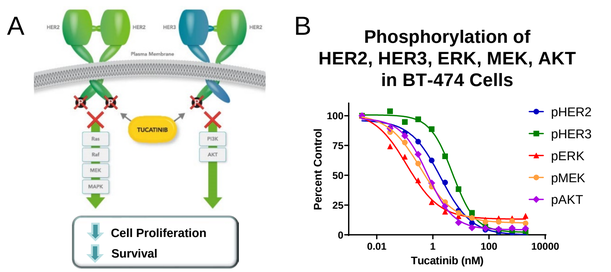


Figure 4. (A) Model of tucatinib-mediated inhibition of HER2 signaling pathways. **(B)** Luminex Assays quantifying phosphorylation of signaling components downstream to HER2 in BT-474 cells. HER2, HER3 (total Tyr), ERK1/2 (Thr185; Tyr187), MEK1 (Ser222) and AKT (Ser473) were measured in cell extracts after 2-hour tucatinib treatment.

Conclusions

- Tucatinib is a HER2-selective TKI with potent anti-tumor activity in breast cancer cell lines, and in vivo in breast cancer xenograft models.
- Tucatinib cytotoxic activity is most often synergistic when combined with T-DM1, with the combination additionally showing enhanced anti-tumor activity in vivo in most xenograft models of HER2⁺ metastatic breast cancer.
- Mechanistically, tucatinib potently suppresses HER2-mediated signaling in vitro as well as in vivo in HER2-overexpressing tumors.
- Tucatinib is detectable in CNS implanted tumors, inhibits growth, and suppresses downstream signaling through the PI3K/AKT pathway, suggesting that therapeutically relevant drug concentrations are achievable.
- These results, taken together with early clinical data demonstrating preliminary safety and activity of tucatinib with T-DM1, support continued assessment of tucatinib in combination with T-DM1 in HER2⁺ MBC patients, including those patients with brain metastases

DISCLOSURES: DJO, AK, JT, MZ, AN, KH, MU, NS, and SRP are employees of Seattle Genetics.

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