Tucatinib potentiates the activity of the antibody-drug conjugate T-DM1 in preclinical models of HER2-positive breast cancer

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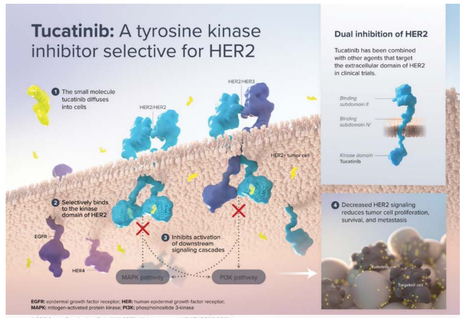
Background

Tucatinib is an orally administered, reversible, highly specific HER2 tyrosine kinase inhibitor recently approved by the FDA in combination with trastuzumab and capecitabine for adult patients with advanced unresectable or metastatic HER2-positive breast cancer (MBC), including patients with brain metastases, that have failed at least one anti-HER2 regimen in the metastatic setting.[1] In a phase IB clinical trial, tucatinib in combination with the HER2-targeted antibody-drug conjugate (ADC) ado-trastuzumab emtansine (T-DM1) was well tolerated and demonstrated activity in heavily pre-pretreated patients with HER2-positive MBC (NCT01983501; Borges VF et al., 2018).[2] We previously presented preclinical data that tucatinib increases the activity of trastuzumab-derived ADCs in HER2-positive breast cancer models.[3,4]

Here, we provide mechanistic insight that tucatinib potentiates the activity of T-DM1 by modulating HER2 protein dynamics and facilitating increased cytotoxic maytansinoid drug delivery.

Proposed mechanism of action for tucatinib[3]



Methods

- To demonstrate the efficacy of tucatinib in combination with T-DM1, cytotoxicity assays were conducted on HER2+ breast cancer cell lines, and in vivo tumor growth studies were conducted on xenograft models.
- To assess changes to HER2 protein levels upon treatment with tucatinib, HER2-amplified breast cancer cell lines were analyzed by Western blot and quantitative FACS (qFACS).
- To probe the dynamics of HER2 at the cell surface upon binding to antibody therapeutics, HER2 internalization assays were conducted over the course of 72 hours.
- To directly measure the rates of ADC catabolism, cell lysates were analyzed by mass spectrometry for the T-DM1 adduct, Lys-MCC-DM1.

Results

The combination of tucatinib and T-DM1 can have increased cytotoxic activity in vitro[4]

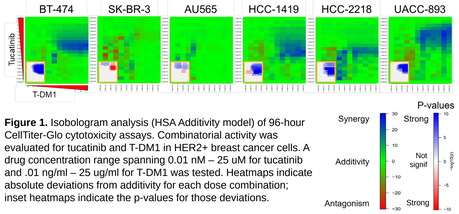
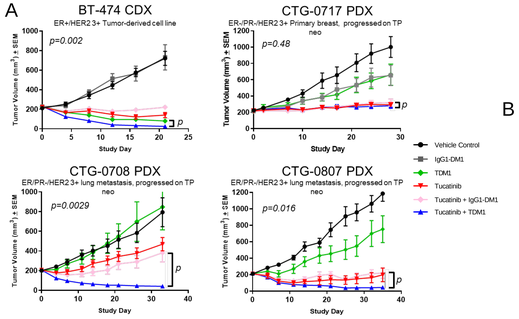


Figure 1. Isobologram analysis (HSA Additivity model) of 96-hour CellTiter-Glo cytotoxicity assays. Combinatorial activity was evaluated for tucatinib and T-DM1 in HER2+ breast cancer cells. A drug concentration range spanning 0.01 nM – 25 uM for tucatinib and .01 ng/ml – 25 ug/ml for T-DM1 was tested. Heatmaps indicate absolute deviations from additivity for each dose combination; inset heatmaps indicate the p-values for those deviations.

Tucatinib/T-DM1 combinatorial activity is observed in vivo in tumor xenograft models[4]



B

Xenograft Model	Tucatinib 50 mg/kg BID	T-DM1 10 mg/kg IV	Tucatinib + T-DM1
BT-474	8/9 PR	9/9 PR	6/9 PR; 3/9 CR
CTG-0717	0	0	0
CTG-0708	2/8 PR	0	6/8 PR; 2/8 CR
CTG-0807	6/8 PR	0	8/8 PR

Figure 2. **(A)** Mean tumor volume over time in HER2+ breast cancer BT-474 cell line derived (CDX) and patient-derived (PDX) xenograft models. Tucatinib was administered at 50 mg/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). **(B)** Summary of partial responses (PR) and complete responses (CR).

Treatment with tucatinib increases overall and membrane-bound HER2 levels

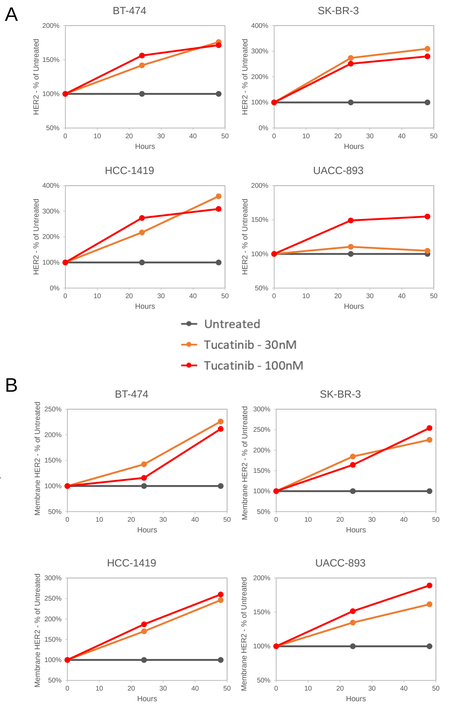


Figure 3. HER2 protein levels were determined for BT-474, SK-BR-3, HCC-1419, and UACC-893 after treatment with tucatinib at either 30nM or 100nM doses, for the duration of 24 hours and 48 hours. **(A)** Total protein levels were determined by western blots using a WES system, and normalized against GAPDH levels as a loading control. **(B)** Plasma membrane-associated levels of HER2 were determined by quantitative FACS (qFACS) analysis.

Increased dwell time of HER2 at the cell surface is followed by rapid internalization and lysosomal processing upon treatment with tucatinib

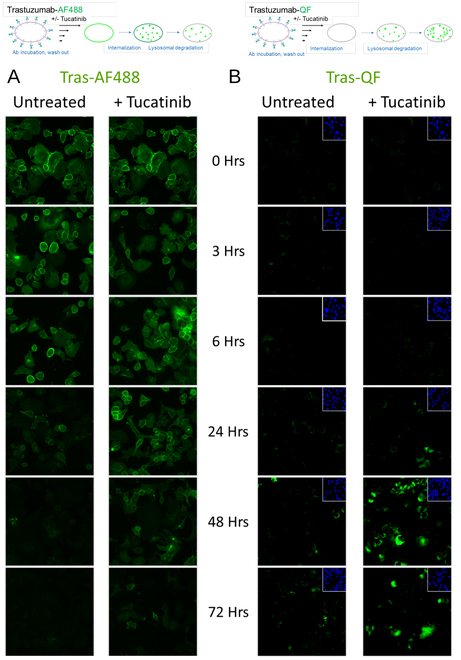


Figure 4. HER2 Internalization assays using Trastuzumab-AF488 **(A)** and Trastuzumab-QF **(B)**. To probe the dynamics of HER2 at the cell surface upon binding to antibody therapeutics in the presence or absence of tucatinib (100nM), SK-BR-3 cells were incubated with fluorescently labeled trastuzumab to mark HER2 at the cell surface. Excess antibody was washed out. Cells were imaged at time points spanning 72 hours to observe internalization of surface-bound antibody. **(B)** Concurrent experiments were conducted with trastuzumab labeled with QF, a fluorophore which is quenched via FRET by Tide Quencher 5WS. Lysosomal cleavage of the linker results in unquenching and fluorescence emission, serving as a proxy for antibody catabolism. Assays were performed in the presence of chloroquine to capture the release of fluorescence.

Tucatinib synergy in combination with T-DM1 correlates with increased intracellular DM1 concentration

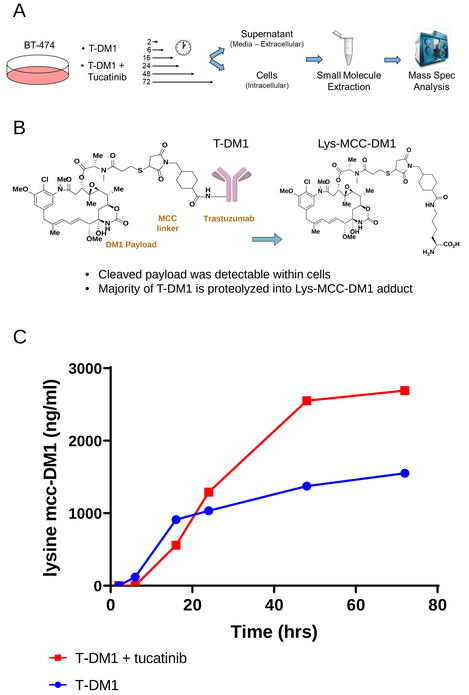


Figure 5. **(A)** Schematic of intracellular drug measurements studies. **(B)** Structure of the primary T-DM1 catabolite, Lys-MCC-DM1, and summary of analysis of adduct studies. **(C)** The concentration of Lysine-MCC-DM1, the primary metabolite of T-DM1, was analyzed over timepoints spanning 72 hours for each treatment condition in lysates of BT-474 cells.

Conclusions

- Tucatinib cytotoxic activity is either additive or synergistic when combined with T-DM1 in vitro in breast cancer cell lines.
- The combination of tucatinib and T-DM1 was more effective in vivo than either single agent alone in BT-474 xenografts and in PDX models tested, producing a higher proportion of partial or complete tumor regressions.
- We present data that provides a mechanistic rationale as to why the co-administration of tucatinib with T-DM1 may be synergistic:
  - In HER2-amplified breast cancer cell lines, treatment with tucatinib increased total and plasma membrane-localized HER2 levels.
  - Treatment with tucatinib had an initial effect that increased the dwell time of HER2 at the cell surface, potentially mediating increased receptor-binding of antibody therapeutics.
  - At later timepoints, HER2 bound to trastuzumab was internalized and directed towards lysosomes.
  - An increased concentration of DM1 adduct was observed by mass spectrometry in tumor cells treated with TDM-1 in combination with Tucatinib, reflecting the internalization kinetics of Trastuzumab.
- The described preclinical in vitro and in vivo data of simultaneous dual HER2 inhibition with tucatinib and T-DM1, taken together with the early clinical data showing preliminary safety and activity of tucatinib with T-DM1, support continued assessment of tucatinib in combination with T-DM1, as well as other HER2-targeted ADCs, in HER2+ metastatic breast cancer patients.

References:
1. TUKYSA [package insert]. Bothell, WA. Seagen Inc., 2020.
2. Borges VF, et al. *Jama Oncol* 2018;4(9):1214-20
3. Kulukian A, et al. *Mol Cancer Ther* 2020;19:976-87
4. Data presented by A. Kulukian, SABCS 2019

DISCLOSURES: AK, JT, DO, MZ, RT, SH, LF, TL, SRP are employees of Seagen. NJ and TP are currently students at Dartmouth College and University of Illinois Urbana-Champaign, respectively.