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Enhancing responses to trastuzumab deruxtecan using tucatinib in HER2-negative breast cancer

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BACKGROUND

- Trastuzumab deruxtecan (T-DXd) is an antibody-drug conjugate (ADC) that has dramatically improved clinical outcomes for patients with metastatic breast cancer compared to traditional systemic chemotherapy
- Response rates in HER2+ disease are robust, however there remains higher variability in response for patients with HER2-negative disease including HER2-low, HER2-ultralow and HER2-0 disease
- Tucatinib is a reversible HER2-directed tyrosine kinase inhibitor which has been shown to increase HER2 protein levels, correlating with increased efficacy of HER2-directed ADCs in HER2+ breast cancer
- **We hypothesized that the addition of tucatinib to T-DXd will enhance its efficacy in HER2-negative breast cancer**

METHODS AND REAGENTS

- **BrC models:** Breast cancer cell lines (BT474, T47D, ZR-75-1, MCF-7, MDA-MB-231) and patient-derived organoids (PDOs, PDO 98 and T638) were maintained in suspension at 37C. Cell lines have been previously authenticated via ATCC
- **FC**: Breast cancer cell lines or patient-derived organoids (PDOs) were isolated and incubated with a FITC-conjugated HER2 antibody for 30 mins. HER2 levels were then quantified by FITC staining intensity using standardized beads as compensation controls. For measurement of T-DXd uptake, the ADC was first incubated with pHrodo Deep Red reagent, cells were then treated with labeled T-DXd for 24 hours at 1 ug/mL. T-DXd uptake was then measured by APC mean fluorescence intensity.
- **Growth Assays:** Breast cancer cells were grown for 5 days and counted every 6 hours via incucyte microscopy or total ATP levels were measured via Cellglo. Media was changed every 24 hours. Cell viability was determined by % increase in cell number compared to vehicle.
- **IHC:** HER2 levels in BC cell lines were independently scored by Dr. Kimberly Cole for comparison with FC-quantified fluorescent intensity
- **Mass spectrometry:** BrC cells were grown for 24 hrs in the presence or absence of T-DXd. Supernatant was collected, cells were washed and then extracted with ice-cold methanol. Dxd was measured via by LC-MS using a Thermo Scientific Vanquish Horizon UHPLC system with a Kinetex F5 column, and a high-resolution Orbitrap IQ-X Tribrid mass spectrometer with a H-electrospray ionization probe operating in positive polarity. Dxd in cell extracts was identified by comparing retention time and characteristic tandem MS/MS fragments compared to pure DXd.
- ***ERBB2* expression quantification:** BrC cells were grown in increasing concentrations of tucatinib for 1-5 days. Total cell RNA was extracted, normalized,and converted to cDNA. Real-time PCR was performed using SYBR® Green Supermix. Relative expression of *ERBB2* was compared to vehicle control conditions on Day 1.

CONCLUSIONS

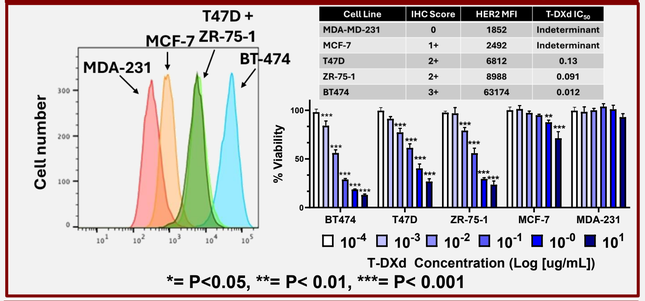
- Tucatinib increased membrane HER2 levels across HER2-negative breast cancer models through increased protein stability
- The addition of tucatinib enhanced T-DXd efficacy in *de novo* resistant cell lines. This correlated with increased T-DXd uptake and deposition of its payload *in vitro.*

FUTURE DIRECTIONS

- Studies are underway determining if the addition of tucatinib enhances the efficacy of T-DXd *in vivo*
- Given the toxicity profile reported using tucatinib + HER2-directed ADCs in patients with HER2+ BrC, alternative combination strategies including tucatinib pulsing are actively being investigated

RESULTS

Fig 1. HER2 MFI correlates with T-DXd efficacy



Cell Line	IHC Score	HER2 MFI	T-DXd IC_{50}
MDA-MD-231	0	1852	Indeterminant
MCF-7	1+	2492	Indeterminant
T47D	2+	6812	0.13
ZR-75-1	2+	8988	0.091
BT474	3+	63174	0.012

*= P<0.05, **= P< 0.01, ***= P< 0.001

Fig 2. Tucatinib increases membrane HER2 levels

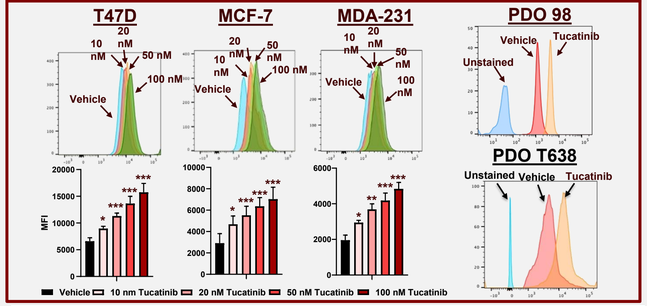


Fig 3. Tucatinib stabilizes HER2 protein

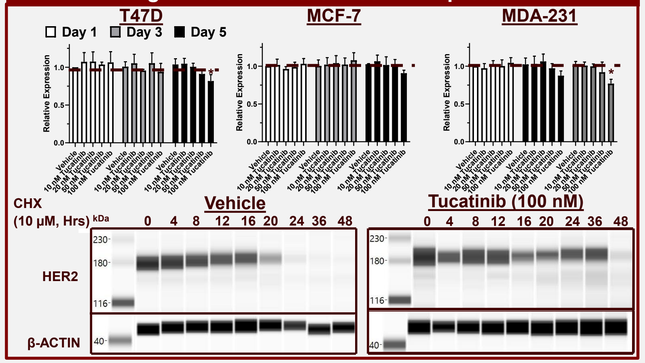
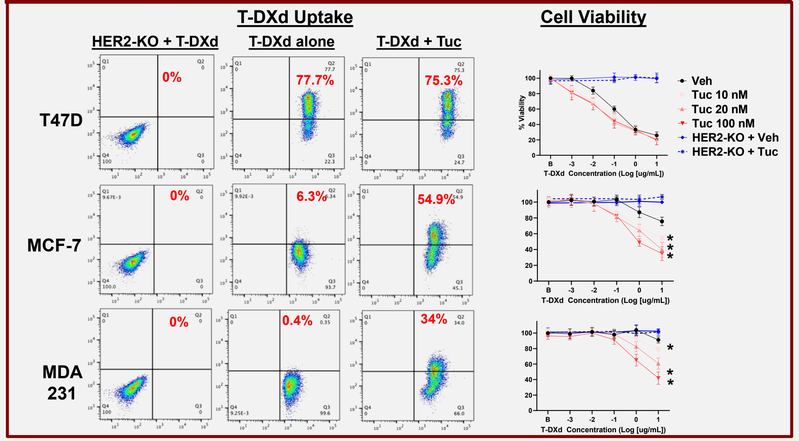
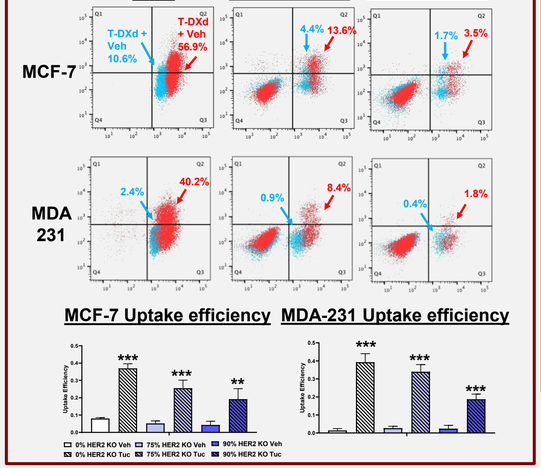


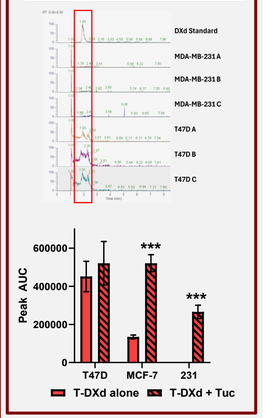
Fig 4. Tucatinib improves T-DXd kinetics and efficacy



T-DXd uptake and efficacy in heterogeneity model



DXd Levels via Mass spec



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