Potential of MEK Inhibition to overcome Trastuzumab deruxtecan Resistance in MAPK-Activated Triple-Negative Breast Cancer PDX Models

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Background

The antibody–drug conjugate (ADC) trastuzumab deruxtecan (T-DXd) has demonstrated remarkable efficacy not only in HER2-positive breast cancer but also in HER2-low disease, including subsets of triple-negative breast cancer (TNBC). However, the mechanisms underlying primary and acquired resistance to T-DXd in TNBC remain poorly defined. To identify predictive biomarkers and rational therapeutic strategies to overcome resistance, we performed targeted proteomic profiling and functional validation studies using a panel of TNBC patient-derived xenograft (PDX) models and cell lines.

Methods and Materials

We performed liquid chromatography–multiple reaction monitoring (LC-MRM) mass spectrometry analysis on tumor lysates from 19 triple-negative breast cancer (TNBC) patient-derived xenograft (PDX) models previously characterized for T-DXd response. The targeted proteomic panel encompassed 91 peptides representing 63 proteins, including HER family receptors, DNA damage response (DDR) components, and key signaling effectors.

To further investigate mechanisms of resistance and therapeutic vulnerabilities, we conducted preclinical studies evaluating the antitumor efficacy of T-DXd and the MEK1/2 inhibitor selumetinib, administered individually or in combination, in HER2-low TNBC PDX models and T-DXd–resistant TNBC cell lines (WHIM3 and WHIM4). Tumor fragments were implanted into the fourth mammary fat pad of recipient mice, and treatments were initiated once tumors reached approximately 250 mm³. Mice were randomized to receive vehicle, T-DXd (1–8 mg/kg, intravenous, once weekly × 1–2 doses), olaparib/selumetinib (both 50 mg/kg, oral, five times per week × 2–3 weeks), or the combinations. Tumor volumes were measured twice weekly until endpoints.

Previous Data

Figure 1. TNBC PDX models WHIM3 (HER2 0) and WHIM4 (HER2 ultra-low) exhibited resistance to T-DXd both as single agent and in combination with the PARP inhibitor olaparib

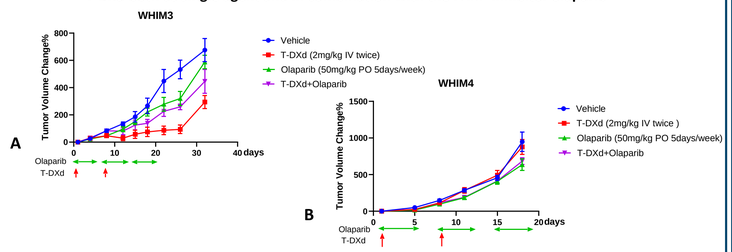
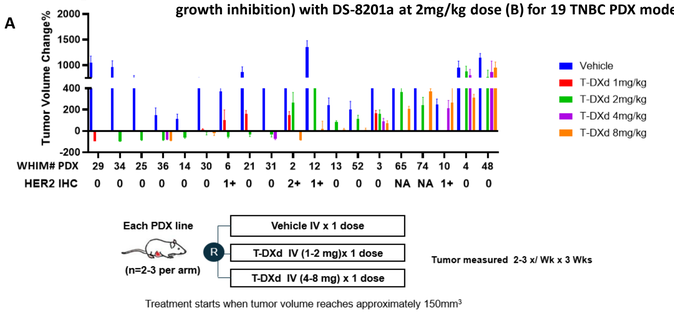


Fig. 1. T-DXd and PARP inhibitor olaparib combination was ineffective to reduce tumor growth in both resistant models WHIM3 (A) and WHIM4 (B). Bidimensional tumor measurement occurred every 2-3 days. Tumor volume (V) was calculated from the equation $V = ab^2/2$, where a and b are tumor length and width (in millimeters), respectively.

Results

Figure 2. % Tumor volume change at week 3-4 following treatment compared to baseline (A) and % TGI (tumor growth inhibition) with DS-8201a at 2mg/kg dose (B) for 19 TNBC PDX models



B

	TGI%
WHIM4	5%
WHIM48	19%
WHIM3	25%
WHIM65	27%
WHIM10**	51%
WHIM13	70%
WHIM12	77%
WHIM2*	77%
WHIM74	78%
WHIM6	90%
WHIM31	101%
WHIM21	102%
WHIM34	109%
WHIM29	111%
WHIM30	114%
WHIM36	124%
WHIM25	128%
WHIM14	146%

Fig. 2. A) 1-8 mg/kg T-DXd treatments revealed which WHIM PDX models were sensitive/resistant. B) Classification, based on %TGI after 2mg/kg T-DXd treatment (except WHIM2/10 ,* 1 mg/kg; ** 4 mg/kg) in 19 TNBC PDX models to establish which one are sensitive/resistant.

Figure 3. Peptides significantly differentially expressed between Sensitive (%TGI >80%) vs Resistant (%TGI < 80%) PDX models using 2mg/kg dosing as reference dose

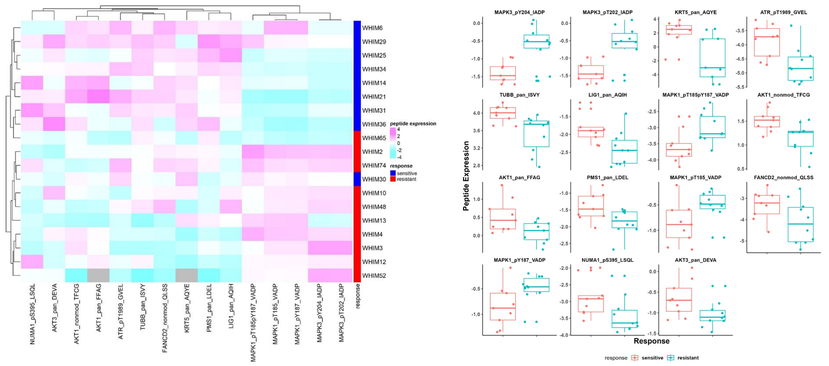


Fig 3. A positive association was observed between T-DXd sensitivity and the proteomic upregulation of KRT5, pATR, AKT1, pAKT1, AKT3, TUBB, LIG1, PMS1, FANCD2, NUMA1. In contrast, T-DXd–resistant models exhibited elevated levels of pMAPK3 and pMAPK1, indicating an inverse correlation between MAPK pathway activation and drug response.

Table 1. Biomarker and key genomic alterations of WHIM3 and WHIM4 PDX models

PDX ID	ER	Her2	Her2 ultra low	Selected Mutated Genes
WHIM3	neg	0	no	KRAS p.G13D; NF1 p.Q1775L
WHIM 4	neg	0	yes	TP53 p.E326Q, p.E285, p.R175H; ALK p.R395H

Figure 4. T-DXd combined with MEK1/2 inhibitor selumetinib was effective in reducing tumor growth in both T-DXd-resistant PDX models

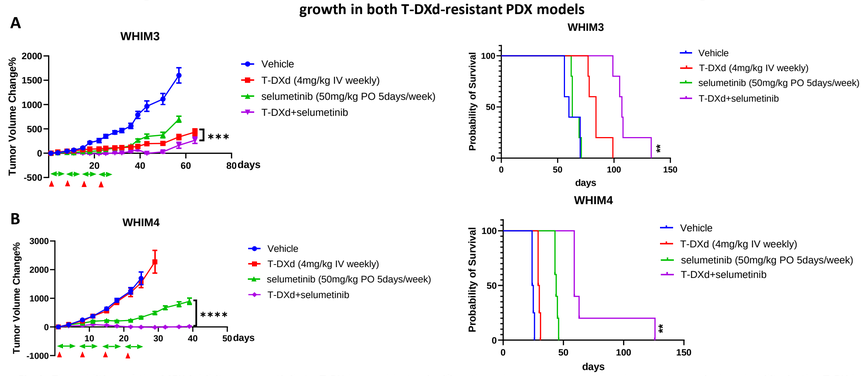


Fig 4 The addition of the MEK inhibitor selumetinib to T-DXd was able to significantly reduce tumor growth and promote survival probability in the T-DXd resistant PDX models WHIM3 (A) and WHIM4 (B), whereas resistance persisted when T-DXd was combined with the DNA damage repair agent olaparib (Figure 1). These findings suggest that inhibition of the MAPK/MEK pathway may overcome T-DXd resistance in TNBC.
Tukey's multiple comparisons test, *** *P value* < 0.001, **** *P value* < 0.0001; Mantel-Cox test, ** *P value* < 0.001.

Figure 5. MEK1/2 inhibitor selumetinib upregulated HER2 expression and the combination with T-DXd suppressed stem cell markers and epithelial–mesenchymal transition (EMT) inducers.

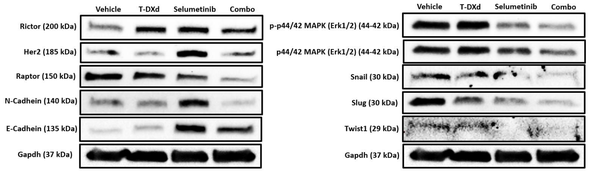


Fig 5. In WHIM3 derived cell line model, the combination of T-DXd and selumetinib was most effective in reducing markers of stem cell and EMT, as well as ERK1/2 signaling, compared to either drug alone. HER2 expression was induced following selumetinib treatment. As expected, T-DXd +/- selumetinib reduced HER2 level.

Conclusions

- **We observed hyperactivation of MAPK pathway in association with T-DXd resistance in TNBC PDX models.**
- **MEK1/2 inhibitor upregulated HER2 expression, and its combination with T-DXd suppressed markers of EMT and stem cell, enhancing the anti-tumor effect of T-DXd in T-DXd resistant HER2 ultra-low and HER2 0 TNBC PDX models.**

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References

1. *Kennedy, J.J. et al. Quantification of Human Epidermal Growth Factor Receptor 2 by Immunopeptide Enrichment and Targeted Mass Spectrometry in Formalin-Fixed Paraffin-Embedded and Frozen Breast Cancer Tissues. Clinical Chemistry 67, 1008-1018 (2021).*
2. Baker A, Wyatt D, Bocchetta M, et al. Notch-1-PTEN-ERK1/2 signaling axis promotes HER2+ breast cancer cell proliferation and stem cell survival. *Oncogene*. 2018;37(33):4489-4504. doi:10.1038/s41388-018-0251-y

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