Presentation Number: PD3-07

Deciphering Resistance Mechanisms to Fam-trastuzumab deruxtecan-nxki in Metastatic Breast Cancer Using Real-World Data

A. A. Potdar[1], Y. Ye[1], P. Sachdev[1], V. Devanarayan[1], Y. Zhang[1]

[1]Clinical Evidence Generation, Eisai Inc., Nutley, NJ

SAN ANTONIO BREAST CANCER SYMPOSIUM®

UT Health San Antonio Mays Cancer Center

AACR American Association for Cancer Research®

Introduction

- Fam-trastuzumab deruxtecan-nxki (T-DXd) has shown clinical benefit in HER2-positive and HER2-low metastatic breast cancer (MBC), but resistance remains a barrier to durable response.
- To investigate resistance mechanisms, real-world data (RWD) from a large, clinically annotated cohort were analyzed to identify molecular correlates and potential therapeutic vulnerabilities.
- This study explores primary (intrinsic) mechanisms and pathways of resistance to T-DXd in HER2-low and HER2-positive patient subgroups using transcriptomics from biopsies prior to T-DXd treatment.

Methods and Materials

- A retrospective analysis of RWD from 300 MBC patients treated with T-DXd (Tempus database[1]) was conducted. Real-world clinical endpoints and baseline tumor transcriptomics prior to T-DXd treatment (Tempus xR[2] RNA-seq) were integrated.
- Patients were stratified as responders (complete or partial response) or non-responders (stable or progressive disease, SD/PD) based on curated real-world best overall response (rwBOR).
- Criteria included: (1) SD ≥ 5 weeks post-treatment; (2) partial response/complete response sustained ≥ 4 weeks without reversion; and (3) manual adjudication of discordant cases with Eisai's translational and clinical teams. Additional endpoints, real-world progression free survival (rwPFS), time to next treatment (rwTTNT), and overall survival (rwOS), were derived using validated Tempus algorithms.
- HER2 status was defined using immunohistochemistry (IHC; 0, 1+, 2+, 3+) and in-situ hybridization (ISH; negative, positive). HER2-low was defined as IHC 1+ or IHC 2+ with a negative ISH, HER2-positive was defined as IHC 2+ with a positive ISH or IHC 3+.
- Genes with expression of < 0.1 TPM (transcripts per million) across > 70% of subjects in cohort were removed from baseline transcriptomics.
- Principal component analysis was used to identify and then remove outlier samples.
- Differential gene expression (DGE) analysis was performed using Limma,[3] adjusting for key covariates (collection site, pre- T-DXd estrogen receptor (ER)/progesterone receptor (PR) status, age at treatment, time/careplan between sample collection and T-DXd start). Cox proportional hazards models were applied to survival endpoints. Gene set enrichment analysis (GSEA)[4] was used to identify biologically relevant pathways.
- Analyses were conducted separately in either HER2-positive or HER2-low subjects.

Results

- After quality control, transcriptomic data were available for 231 patients with matched rwBOR (120 non-responders, 111 responders) and survival endpoints were evaluable in 230 patients (**Table 1**).

Largely non-overlapping resistance-associated genes between HER2-positive and HER2-low subgroups

- DGE analysis revealed largely non-overlapping resistance-associated genes between HER2-positive and HER2-low subgroups, suggesting distinct resistance mechanisms.
- Similar observation was noted using other clinical endpoints (**Table 2**, **Figure 1**).

Gene-level correlates of real-world survival endpoints (HER2-positive)

- Survival analysis identified 383 genes in HER2-positive, significantly associated with either of the 3 real-world survival endpoints (q < 0.2; HR > 2 or HR < 0.5).
- Survival analysis identified 45 genes in HER2-positive, significantly associated with either rwPFS (q < 0.2; HR > 2 or HR < 0.5) or both other real-world survival endpoints (rwOS and rwTTNT, q < 0.2; HR > 2 or HR < 0.5).
- Notable top genes among above (by q value) associated with at least 2 survival endpoints included *TMEM150A*, *DENND2D*, and *SLC6A14* (**Figure 2**) with roles implicated in membrane transport and metabolic reprogramming.

Gene-level correlates of real-world survival endpoints (HER2-low)

- Survival analysis identified 47 genes in the HER2-low subgroup, significantly associated with either of the 3 real-world survival endpoints (q < 0.2; HR > 2 or HR < 0.5).
- Survival analysis identified 29 genes in the HER2-low subgroup, significantly associated either rwPFS (q < 0.2; HR > 2 or HR < 0.5) or both other real-world survival endpoints (rwOS and rwTTNT, q < 0.2; HR > 2 or HR < 0.5).
- Notable top genes among above (by q value) associated with at least 2 survival endpoints included *C1ORF61* and *SERTAD1* (**Figure 3**) with roles implicated in transcriptional regulation and cell cycle modulation.

Table 1. Sample size for integrated real-world clinical endpoints and baseline tumor transcriptomics dataset available including key covariates. See Poster #PS2-10-24 for demographic and other key characteristics for MBC 300 cohort.

Characteristic	rwBOR		rwPFS		rwTTNT		rwOS	
	Non-responder N = 120	Responder N = 111	Censored N = 40	Event N = 190	Censored N = 112	Event N = 113	Censored N = 126	Event N = 104
Mean age at T-DXd, years (SD)	58 (13)	58 (14)	63 (11)	57 (13)	59 (13)	57 (13)	58 (13)	58 (13)
ER.Tempus.final, n (%)								
ER+	77 (64)	74 (67)	27 (68)	124 (65)	78 (70)	69 (61)	83 (66)	68 (65)
Other	43 (36)	37 (33)	13 (33)	66 (35)	34 (30)	44 (39)	43 (34)	36 (35)
PR.Tempus.final, n (%)								
PR+	41 (34)	46 (41)	17 (43)	69 (36)	46 (41)	38 (34)	55 (44)	31 (30)
Other	79 (66)	65 (59)	23 (58)	121 (64)	66 (59)	75 (66)	71 (56)	73 (70)
careplan.between.RNA.and.T-DXd, n (%)	99 (83)	90 (81)	31 (78)	157 (83)	92 (82)	92 (81)	97 (77)	91 (88)
Mean time.RNA.before.T-DXd, days (SD)	579 (687)	484 (563)	575 (655)	534 (635)	555 (613)	538 (674)	521 (615)	565 (665)
HER2 status, n (%)								
HER2-Positive	30 (25)	47 (42)	18 (45)	58 (31)	41 (37)	32 (28)	49 (39)	27 (26)
HER2+/Low	9 (7.5)	3 (2.7)	0 (0)	12 (6.3)	5 (4.5)	6 (5.3)	3 (2.4)	9 (8.7)
HER2-Low	61 (51)	49 (44)	16 (40)	94 (49)	51 (46)	58 (51)	58 (46)	52 (50)
HER2-/Low	10 (8.3)	4 (3.6)	1 (2.5)	13 (6.8)	5 (4.5)	9 (8.0)	5 (4.0)	9 (8.7)
HER2-	10 (8.3)	6 (5.4)	3 (7.5)	13 (6.8)	8 (7.1)	8 (7.1)	9 (7.1)	7 (6.7)
Unknown	0 (0)	2 (1.8)	2 (5.0)	0 (0)	2 (1.8)	0 (0)	2 (1.6)	0 (0)
RNA.Biopsy.site.final, n (%)								
Breast	41 (34)	39 (35)	18 (45)	62 (33)	43 (38)	36 (32)	51 (40)	29 (28)
Liver	27 (23)	28 (25)	8 (20)	47 (25)	30 (27)	24 (21)	32 (25)	23 (22)
Other	52 (43)	44 (40)	14 (35)	81 (43)	39 (35)	53 (47)	43 (34)	52 (50)

HER2+/Low – HER2 IHC 2+, missing FISH; HER2-/Low – HER2 IHC numerical missing, categorical is Negative.

Table 2. Number of associated genes with a real-world endpoint using various significance criteria.

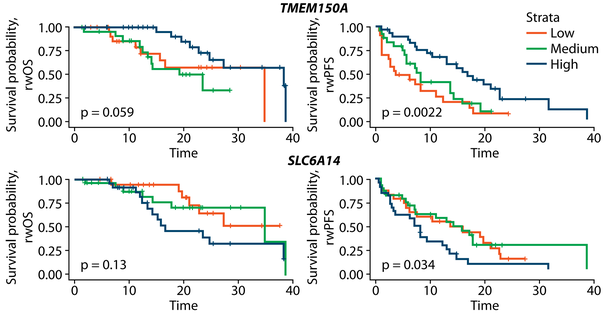
Real-world Endpoint	HER2 Subgroup	P < 0.05	Number FDR < 0.2	Number |CohensD| > 0.8	Number FDR < 0.2 and |CohensD| > 0.8
rwBOR (NR vs R)	HER2Low	1158	15	173	2
rwBOR (NR vs R)	HER2pos	1340	8	352	5
Real-world endpoint	**HER2 Subgroup**	**P < 0.05**	**Number FDR < 0.2**	**Number HR > 2 or HR < 0.5**	**Number FDR < 0.2 and HR > 2 or HR < 0.5**
rwOS	HER2Low	1734	28	743	20
rwOS	HER2pos	2086	299	3029	243
rwPFS	HER2Low	2041	86	242	29
rwPFS	HER2pos	1618	73	786	42
rwTTNT	HER2Low	782	1	361	1
rwTTNT	HER2pos	1914	157	2166	110

CohensD, Standardized effect size; FDR, false discovery rate; HR, Hazard ratio; NR, non-responder; R, responder.

Figure 1. Venn diagrams indicating overlap of genes between HER2-positive and HER2-low subgroups using A. |CohensD|> 0.8 criteria in NR vs R, DGE analyses using rwBOR B. HR > 2.0 or HR < 0.5 in survival analysis using rwOS.

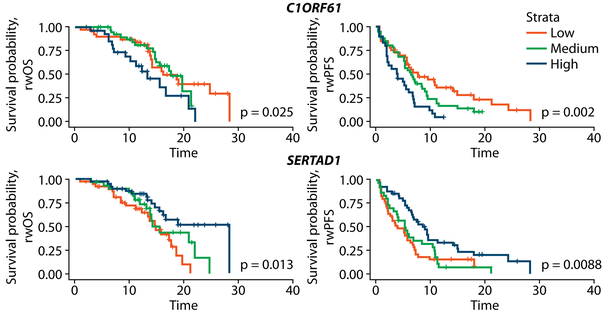


Figure 2. Kaplan-Meier plots of *TMEM150A* (top) and *SLC6A14* (bottom) with rwPFS and rwOS endpoints. Within **HER2-positive subgroup**, higher TMEM150A expression is associated with favorable outcomes while higher SLC6A14 expression is associated with worst outcomes.



K-M plots for select genes; P values if not specified are from log-rank tests.

Figure 3. Kaplan-Meier plots of *C1ORF61* (top) and *SERTAD1* (bottom) with rwPFS and rwOS endpoints. Within **HER2-low subgroup**, higher *C1ORF61* expression is associated with worst outcomes while higher *SERTAD1* expression is associated with favorable outcomes.

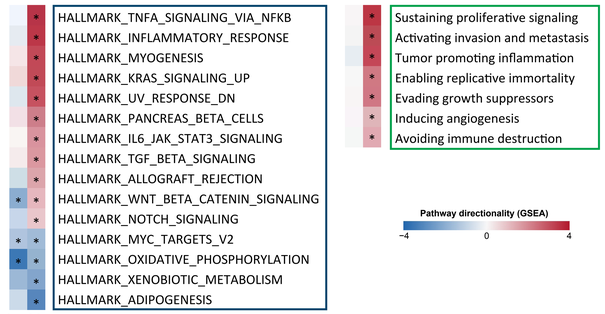


K-M plots for select genes; P values if not specified are from log-rank tests

Distinct mechanisms of T-DXd primary resistance between HER2-positive and HER2-low

- In HER2-low non-responders, significant enrichment (q < 0.2) was observed in multiple oncogenic pathways including TNF/NF-κB, TGF-β, KRAS, protein signaling and inflammatory response – implicated in immune evasion, epithelial-mesenchymal transition (EMT), and resistance to antibody-drug conjugates (see **Figure 4**). In contrast, HER2-positive non-responders showed negative enrichment of Wnt/β-catenin signaling and oxidative phosphorylation and, suggesting metabolic reprogramming.
- Several cancer hallmark pathways such as sustaining proliferative signaling, activating invasion and metastasis, and tumor promoting inflammation were significantly positively enriched (q < 0.2) in non-responders within HER2-low subgroup.
- These results indicate that primary T-DXd resistance mechanisms may be distinct within HER2-low and HER2-positive subgroups.

Figure 4. Comparison of T-DXd primary resistance mechanisms in **A. HER2-positive** (NR, n = 30 vs R, n = 47) and **B. HER2-low** subgroups (NR, n = 61 vs R, n = 49).



*q < 0.2.

Increased pathway activity indicated by red cells while decrease indicated by blue cells as determined by gene set enrichment analysis (GSEA). Pathways sorted by decreasing enrichment in HER2-low, NR vs R (q < 0.2) within each of the gene set categories (Hallmark gene sets from Molecular Signatures Database (in blue box) and Cancer Hallmark (green box) gene sets).

Conclusions

- This study represents one of the largest real-world transcriptomic analyses of resistance in T-DXd-treated MBC.
- There are distinct signaling profiles associated with response to T-DXd between HER2-positive and HER2-low metastatic breast tumors, highlighting biological heterogeneity of resistance.
  - In HER2-positive patients, T-DXd efficacy appears to be primarily driven by HER2 signaling, with limited activation of alternative resistance pathways.
  - In contrast, HER2-low non-responders show activation of multiple oncogenic pathways, suggesting that resistance to T-DXd in these tumors is driven by diverse mechanisms not observed in HER2-positive non-responders.
- Enrichment of immune and EMT pathways in HER2-low non-responders suggests potential benefit from combination strategies, such as immune-checkpoint inhibitors or pathway-targeted agents.
- Gene-level correlates of real-world endpoints support biomarker-driven therapeutic optimization and warrant prospective validation to inform future resistance-mitigation strategies in T-DXd-treated MBC.

Funding statement: Study sponsored by Eisai Inc., Nutley, NJ, USA.

Corresponding authors: Alka A. Potdar, e-mail: Alka_Potdar@eisai.com; Yan Zhang, e-mail: Yan_Zhang@eisai.com

References: 1. www.tempus.com; **2.** Tempus-xR_Validation.pdf; **3.** Ritchie, Matthew E., et al., Nucleic Acids Research 43.7 e47-e47 (2015); **4.** Subramanian, Aravind, et al., Proceedings of the National Academy of Sciences 102.43 15545-15550 (2005).

Copies of this poster obtained through Quick Response (QR) Code are for personal use only and may not be reproduced without permission from SABCS® and the author.