Mechanisms of Resistance to Trastuzumab Deruxtecan in Breast Cancer Elucidated by Multi-omic Molecular Profiling

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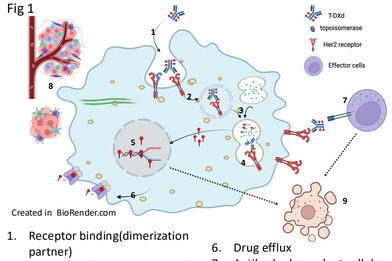
Background

Trastuzumab deruxtecan (T-DXd) is widely used as a treatment for metastatic HER-2 low and HER2-positive breast cancer, and preclinical studies have suggested multiple potential mechanisms of resistance. There have, however, been few large population-based studies of resistance.

Following the course of trastuzumab deruxtecan through the cancer cell (Fig 1), we combined Next Generation Sequencing (NGS), immunohistochemistry (IHC) and claims data to evaluate clinically relevant mechanisms of resistance to this agent.

Fig 1



1. Receptor binding(dimerization partner)
2. Internalization and endocytosis
3. Intracellular trafficking
4. Lysosomal cleavage
5. Topoisomerase inhibition
6. Drug efflux
7. Antibody-dependent cellular cytotoxicity (ADCC)
8. Tumor immune microenvironment
9. Cell death

Methods

- A total of 2799 T-DXd-treated breast cancer samples tested at Caris Life Sciences (Phoenix, AZ) underwent whole transcriptome sequencing (Illumina, NovaSeq).
- Her2 status was determined by Her2 IHC (4B5) and Her2 CISH (INFORM HER-2 Dual ISH DNA Probe) when appropriate; all scores follow ASCO/CAP guidelines.
- Real-world clinical data were obtained from insurance claims. Treatment-associated overall survival (OS) were determined as the interval from the T-DXd initiation to the end of treatment and the last recorded clinical activity, respectively.
- RNA expression of 86 genes linked to numerous reported ADC resistance mechanisms and 11 RNA-based immune signatures were investigated for association with clinical outcome, including
  - Antigen/dimerization partners (N=5),
  - ABC transporters (N=38),
  - Lysosomal genes (N=16),
  - Genes involved in ADCC pathway (N=9),
  - endocytosis, intracellular trafficking and cytoskeleton organization pathways (N=6),
  - topoisomerase isoforms (N=4),
  - tubulin isoforms (N=5) and
  - well-established prognostic markers in breast cancer (N=3).
  - Interferon-gamma signature (N=1) and RNA Deconvolution (QuanTISeq) (N=10)
- Multivariate analysis was conducted using RNA transcript per million (TPM) inputs as continuous variables in a Cox-Proportional Hazard model, with significance determined at p<0.05.
- Molecular comparison were done using Chi-square, p values were adjusted for multiple comparison using Benjamini-Hochberg (q<0.05)

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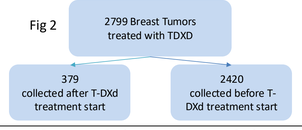
Results

1. Patient characteristics
Consort diagram and patient subtype distribution (Fig2)

Fig 2

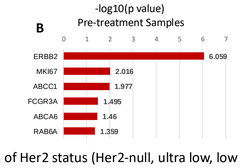


	Category	Post-treatment Specimens	Pretreatment Specimen	Entire Total
Her2 Status	Her2-null	68 (17.9%)	436 (18.0%)	504 (18.0%)
	Her2 Ultra Low	33 (8.7%)	415 (17.1%)	448 (16.0%)
	Her2 Low	142 (37.5%)	812 (33.6%)	954 (34.1%)
	Her2 Positive	50 (13.2%)	396 (16.4%)	446 (15.9%)
	Her2-other/unknown	86 (22.7%)	361 (14.9%)	447 (16.0%)
HR Status	HR Negative	95 (25.1%)	595 (24.6%)	690 (24.7%)
	HR Positive	202 (53.3%)	1502 (62.1%)	1704 (60.9%)
	HR unknown	82 (21.6%)	323 (13.3%)	405 (14.5%)
	Total	379 (100.0%)	2420 (100.0%)	2799 (100.0%)

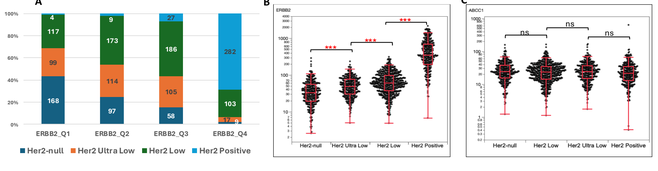
2. Multivariate analysis using Cox-Proportional Regression Model in pre-treatment specimens identifies ABCC1 as highly associated with T-DXd associated OS. (A): top five genes (TPM) unit hazard ratio and FDR p value. (B) Logworth (ie –log10(p value) of the top five genes

A	Unit Hazard Ratio	Lower 95%	Upper 95%	FDR Pvalue (ie, q value)
ERBB2	0.998814	0.99841	0.999218	0
MKI67	1.00522	1.002467	1.00798	0.00964
ABCC1	1.009202	1.004173	1.014256	0.01054
FCGR3A	0.996391	0.994196	0.998592	0.03197
ABCA6	1.039368	1.014489	1.064857	0.03465
RAB6A	1.001145	1.000397	1.001894	0.04374

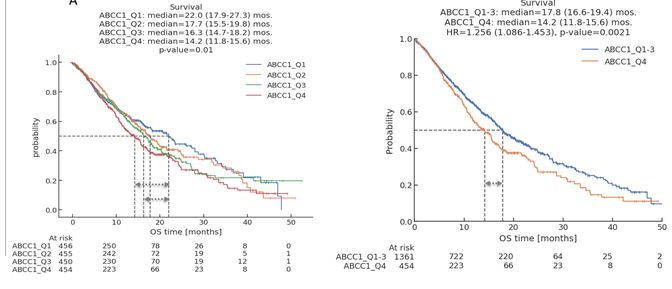
B



3. ERBB2 and ABCC1 expression with Her2 Status. (A) Distribution of Her2 status (Her2-null, ultra low, low and positive categories) in ERBB2 quartiles (Q4: highest expression, Q1: lowest expression). (B) ERBB2 expression (TPM) significantly associate with Her2 status while (C) ABCC1 expression is independent of Her2 status.



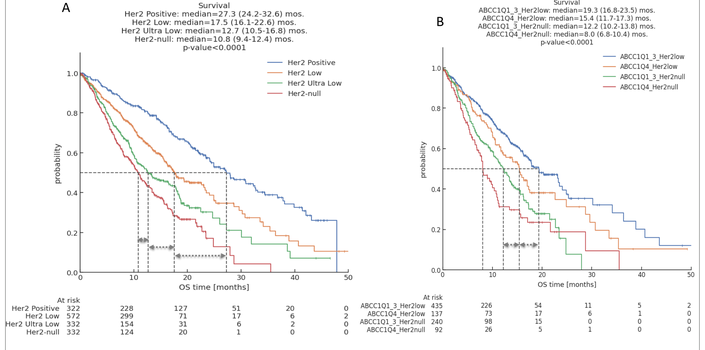
4. T-DXd associated overall survival based on ABCC1 expression (A) ABCC1 quartiles (Q4: highest expression, Q1: lowest expression) and (B) ABCC1 high (Q4) vs. ABCC1 low (Q1-Q3)



Results

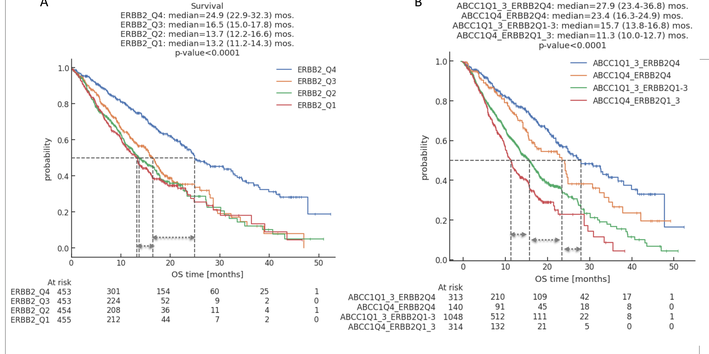
5. T-Dxd associated survival based on Her2 status (A) and a combination of Her2 status and ABCC1 expression (B):

- Patients' Her2 status (Positive, Low, Ultra Low and Null) were determined with a combination of IHC and CISH. Significant difference of T-DXd associated survival was seen between all groups considered
- ABCC1 expression (high: Q4; low: Q1-Q3) further stratifies Her2-null and Her2-low patients' survival. Numerical differences seen in Her2-positive patients but effect not significant (data not shown)



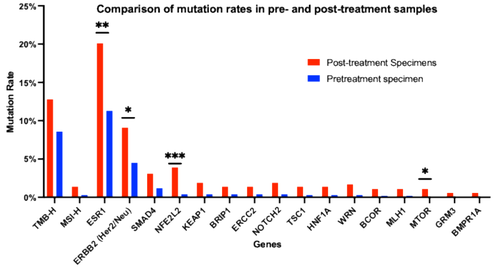
6. T-Dxd associated survival based on Her2 RNA expression (TPM) (A) and a combination of Her2 expression and ABCC1 expression (B):

- patients stratified by ERBB2 expression quartiles (Q4: highest expression, Q1: lowest expression). Significant difference of T-DXd associated survival was seen between all groups considered with Q4 tumors showing the longest survival
- ABCC1 expression (high: Q4; low: Q1-Q3) further stratifies Her2 Q4 and Her2 Q1-3 patients' survival

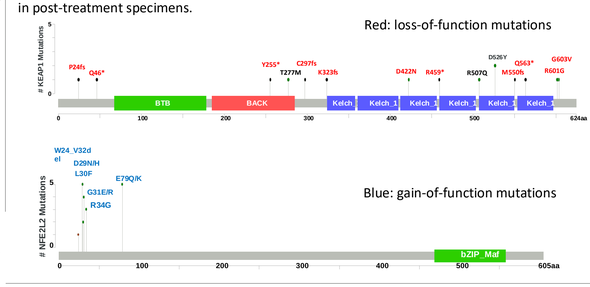


Results

7. Comparison of pre and post treatment molecular profiles. All mutations shown have p<0.05 (unadjusted p value). *: q<0.05; **: q<0.005; ***: q<0.0005



Gain-of-function mutations of NRF2 and loss-of-function mutations of KEAP1 that both lead to deregulation of NRF2/KEAP1 pathway and therefore ABCC1 overexpression are more prevalent in post-treatment specimens.



Conclusions

- Using a multi-omic approach, we thoroughly surveyed gene expressions on pathways associated with antibody-drug conjugate resistance mechanisms.
- Using clinical outcome data from 2799 breast cancer patients treated with T-DXd, we show that clinical outcome is a function of trastuzumab target expression and expression of the key drug efflux pump ABCC1. At an intracellular level these translate to concentration X time for deruxtecan.
- Statistically significant differences were seen comparing post-therapy vs. pre-therapy TDX-d specimens, including ERBB2 somatic mutation as well as mutations known to alter expression of ABCC1 efflux pump (ie KEAP1/NFE2L2)