Abstract 1212
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Molecular Characterization of Resistance to Antibody Drug Conjugates in Metastatic Breast Cancer: A Prospective Analysis from the AURORA US Network

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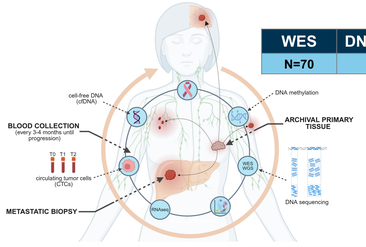
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Background

- With antibody drug conjugates (ADC) as treatment of metastatic breast cancer (mBC), it is key to understand the mechanisms that drive resistance to inform optimal ADC selection and sequencing.
- Preliminary data suggest that ADC resistance may be mediated by different mechanisms involving the payload (e.g., TOP1 mutations after topoisomerase I-inhibitor (TOP1i) ADCs), target (e.g., *TACSTD2* mutations leading to decreased TROP2 expression and ADC binding) or lysosomal processing, among others.[1-4]
- The AURORA (AUR) US Network was established to characterize paired primary and metastatic (met) samples via multiplatform profiling,[5] with a prospective phase of met tissue collection that included pre- and post-ADC samples.

Methods

- Fresh frozen or FFPE met and archival primary tumors were analyzed across 3 platforms: DNA low pass whole genome and exome sequencing (WES), DNA methylation arrays (Methyl) and RNA sequencing (RNA seq). Pairwise gene expression comparisons were performed in R using t-tests, and pathway analysis with GSEA.



WES	DNA Methyl	RNA seq
N=70	N=62	N=69

Patients with any ADC for mBC	N=72
Median age at mBC diagnosis (range)	55.5 (27.7-73.4)
Germline *BRCA1/BRCA2*	9 (12.5%)
De novo mBC	16 (22.2%)
AUR met clinical subtype*	
HR+/HER2-	41 (56.9%)
HR+/HER2+	8 (11.1%)
HR-/HER2+	3 (4.2%)
TNBC	19 (26.4%)
Unknown	1 (1.4%)
Median n chemotherapy for mBC pre-ADC$_1$ (range)	1 (0-4)

*AUR met subtype determined using clinical IHC/FISH data when available; if not available, inferred from RNA-seq data.

	ADC$_1$	ADC$_2$	ADC$_3$
	N=72	N=23*	N=4
HER2	35 (48.6%)	12 (52.2%)	3 (75.0%)
T-DXd	28	12	3
T-DM1	7	-	-
TROP2	36 (50.0%)	11 (47.8%)	1 (25.0%)
SG	35	11	1
Dato-DXd	1	-	-
NECTIN4	1 (1.4%)	-	-
Median duration, days (range)	141 (6-522)	81 (7-425)	89.5 (70-133)
Discontinuation due to PD	58 (80.6%)	18 (78.3%)	4 (100.0%)

*23 pts received ≥ 2 ADC: 16 immediate sequence; 7 intervening therapy PD, progressive disease.

Results

Figure 1. Heatmap of target genes in AUR met. 115 samples (72 pts) were profiled: 91 pre-ADC$_1$ (42 primary, 49 met), 24 post-ADC$_1$ (met) including 5 pre-ADC$_2$ samples. Heatmap of ADC target gene expression in met samples is shown below.

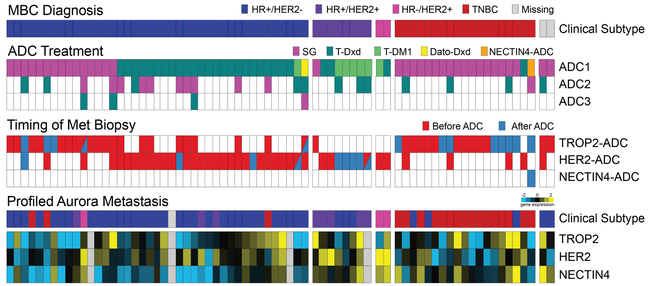
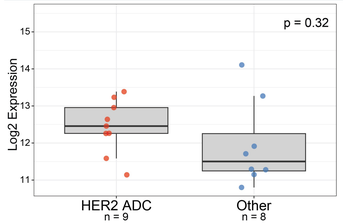


Figure 2. ADC target gene expression in post-treatment samples. *ERBB2* did not differ between samples after HER2 (n=9) vs non-HER2 ADC (n=8) (p=0.32), nor did *TACSTD2* after TROP2 (n=8) vs non-TROP2 ADC (n=9) (p=0.47).

ERBB2 Post-HER2 vs Non-HER2 ADC



TACSTD2 Post-TROP2 vs Non-TROP2 ADC

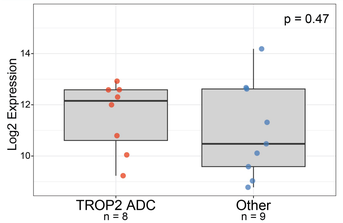
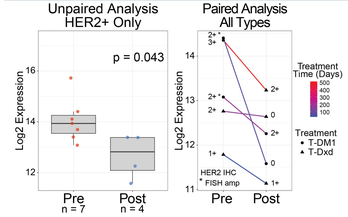
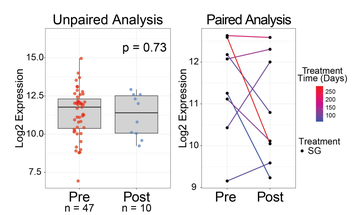


Figure 3. ADC target gene expression pre- vs post-ADC. In pts with HER2+ AUR met who received HER2 ADC, *ERBB2* decreased post (n=4) vs pre-treatment (n=7) (p=0.043). *ERBB2* downregulation was observed in 5 pts with paired pre- vs post-HER2 ADC samples. In pts who received TROP2 ADC, *TACSTD2* did not differ post- (n=10) vs pre-treatment (n=47) (p=0.73).

ERBB2 Pre- vs Post-HER2 ADC



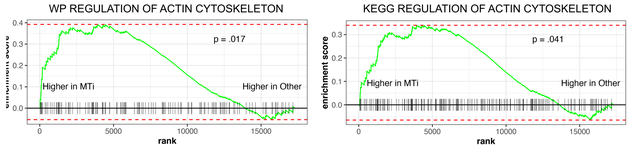
TACSTD2 Pre- vs Post-TROP2 ADC



Results (cont.)

Figure 4. GSEA revealed significant differences in payload-related pathways in post-ADC samples, including actin cytoskeleton in post-microtubule inhibitor (MTi, n=7) vs non-MTi ADC (n=9) samples (WP p=0.017; KEGG p=0.041; padjs <0.225), and DNA damage down (p=1.5e-6), DNA damage up (p=3.1e-4) and DNA damage by gamma and UV radiation (p=2.5e-4) post-TOP1i (n=9) vs pre-ADC (n=83) samples (padjs <0.005). **Lysosomal pathways** did not differ by MTi, TOP1i, or any ADC.

Cytoskeleton Pathways in Post-MTi ADC Samples



DNA Damage and Lysosomal Pathways in Post-TOP1i ADC Samples

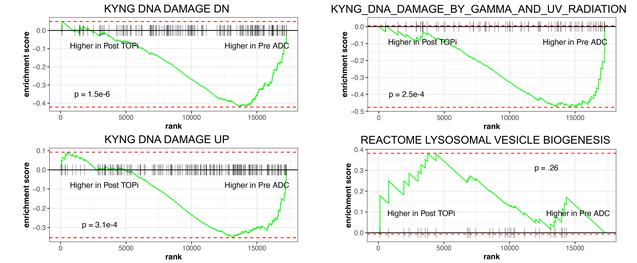


Figure 5. ADC target promoter methylation. *TACSTD2* promoter methylation was observed in 9 pre-ADC samples (median β value >0.3). Promoter methylation of *ERBB2, TACSTD2,* or *NECTIN4* was not detected in any post-ADC samples.

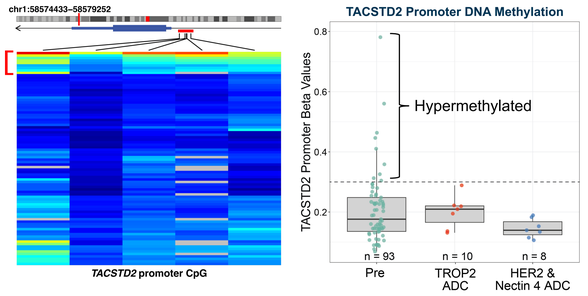
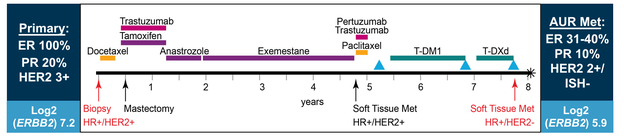
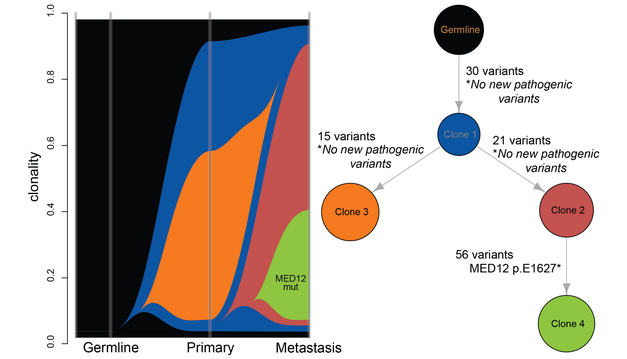


Figure 6. *MED12* LOF mutation post-T-DXd. *MED12* LOF variant (detected in one patient immediately post-T-DXd) has been reported to promote PARP inhibitor and platinum resistance in BRCA-deficient tumors and may represent a novel mechanism of resistance to TOP1i.[6] Red arrows: profiled tumors. Blue triangles: recurrences.





ERBB2, *TACSTD2* or *NECTIN4* mutations were not detected in any post-ADC samples.

Conclusions

- Multiplatform molecular characterization of mBC treated with ADCs revealed potential mechanisms of resistance related to downregulation of target expression and payload (e.g., cytoskeleton, DNA damage repair pathways).
- Additional analyses integrating these multimodal data are ongoing. Strategies to overcome resistance, e.g. modifying ADC target, are being evaluated in clinical trials (e.g., TBCRC064: NCT06533826).

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Acknowledgements

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