CHARISMA: Paired-biopsy multi-omic CHAacterization reveals target-independent ResISStance Mechanisms to Antibody-drug conjugates

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DECLARATION OF INTERESTS

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I have the following financial relationships to report:

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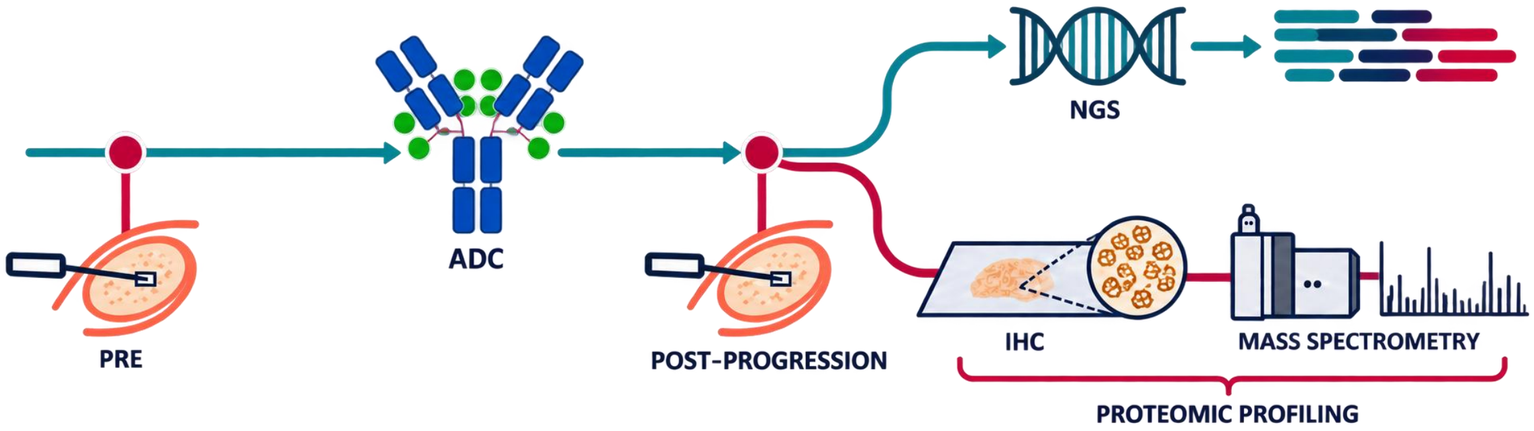
AstraZeneca, BioNTech, Boehringer Ingelheim, Daiichi Sankyo, Gilead, Roche/Genentech, Eli Lilly, Merck, Menarini/Stemline, Novartis, Sanofi, Servier

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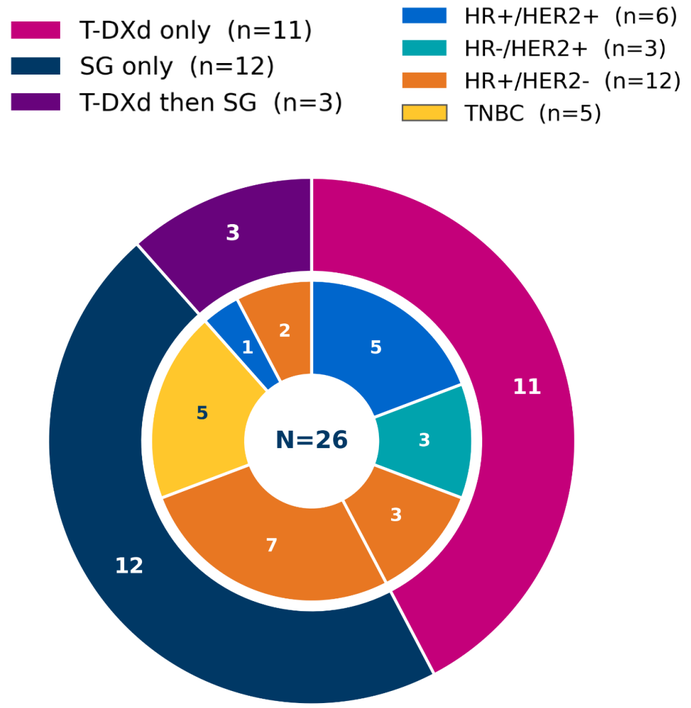
Background and Methods

- Mechanisms of **resistance to TOPO1i ADCs** remain poorly understood.
- We conducted **multiomic profiling of paired biopsies from 26 patients** with **metastatic breast cancer**, collected before and after **T-DXd (n=11), SG (n=12),** or sequential **T-DXd followed by SG (n=3).**



Real-world cohort: biopsy sites and intervening therapies varied.

Cohort (n=26)



Number of samples with data available varied across different techniques. No samples were excluded due to non-QC reasons

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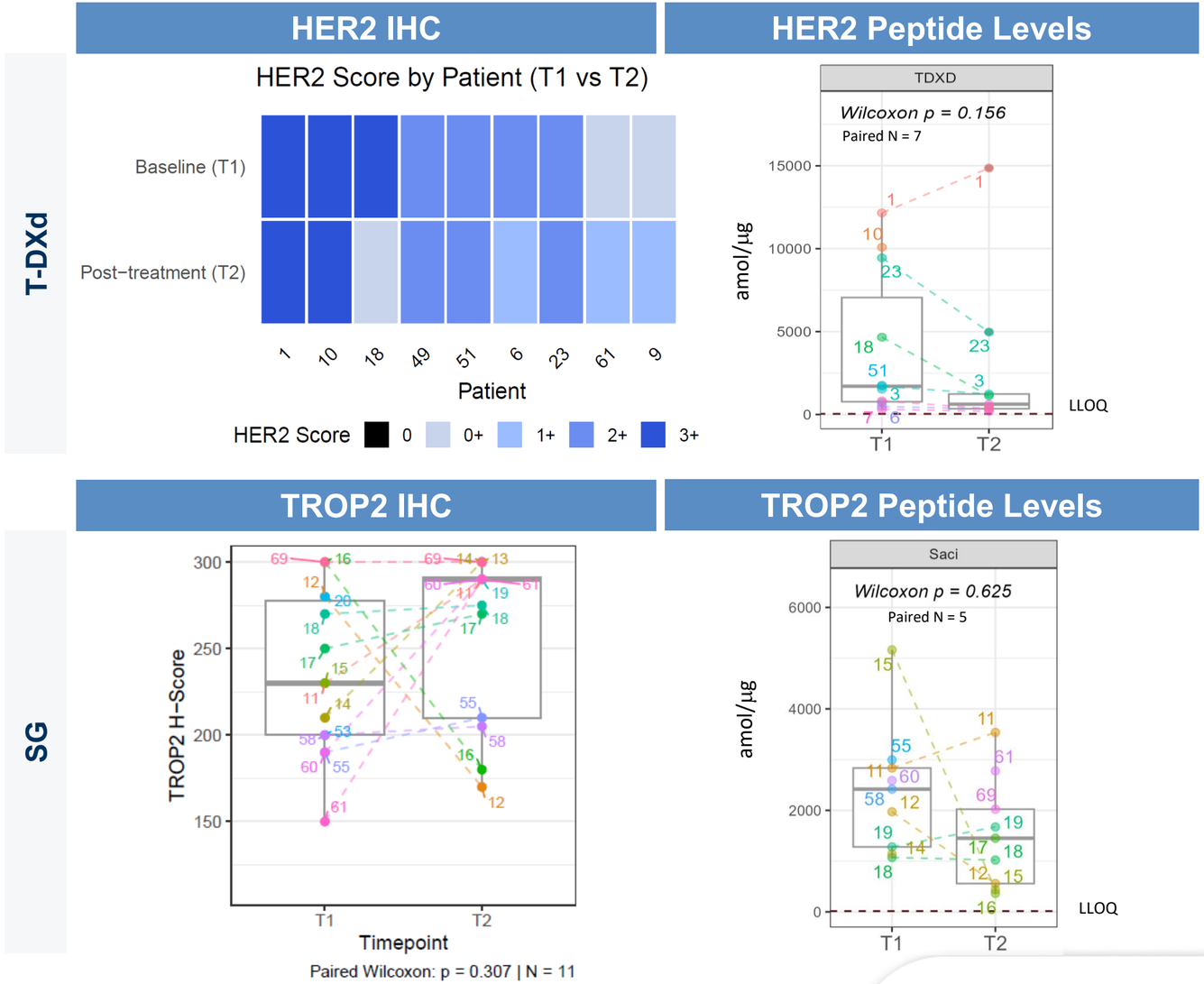
ADC (antibody-drug conjugate); T-DXd (trastuzumab deruxtecan); SG (sacituzumab govitecan), TOP1i (topoisomerase 1 inhibitors)

ADC Target Expression can Decrease at Progression, yet Usually Remains Detectable

- **HER2 decreased in 8/14 (57%) after T-DXd** by IHC and/or MS.



- However, **some degree of HER2 expression remained detectable in all tumors** by IHC and/or MS.
- After **SG**, there was **no consistent TROP2 downregulation** (5/14 with TROP2 increase, 3/14 with decrease).



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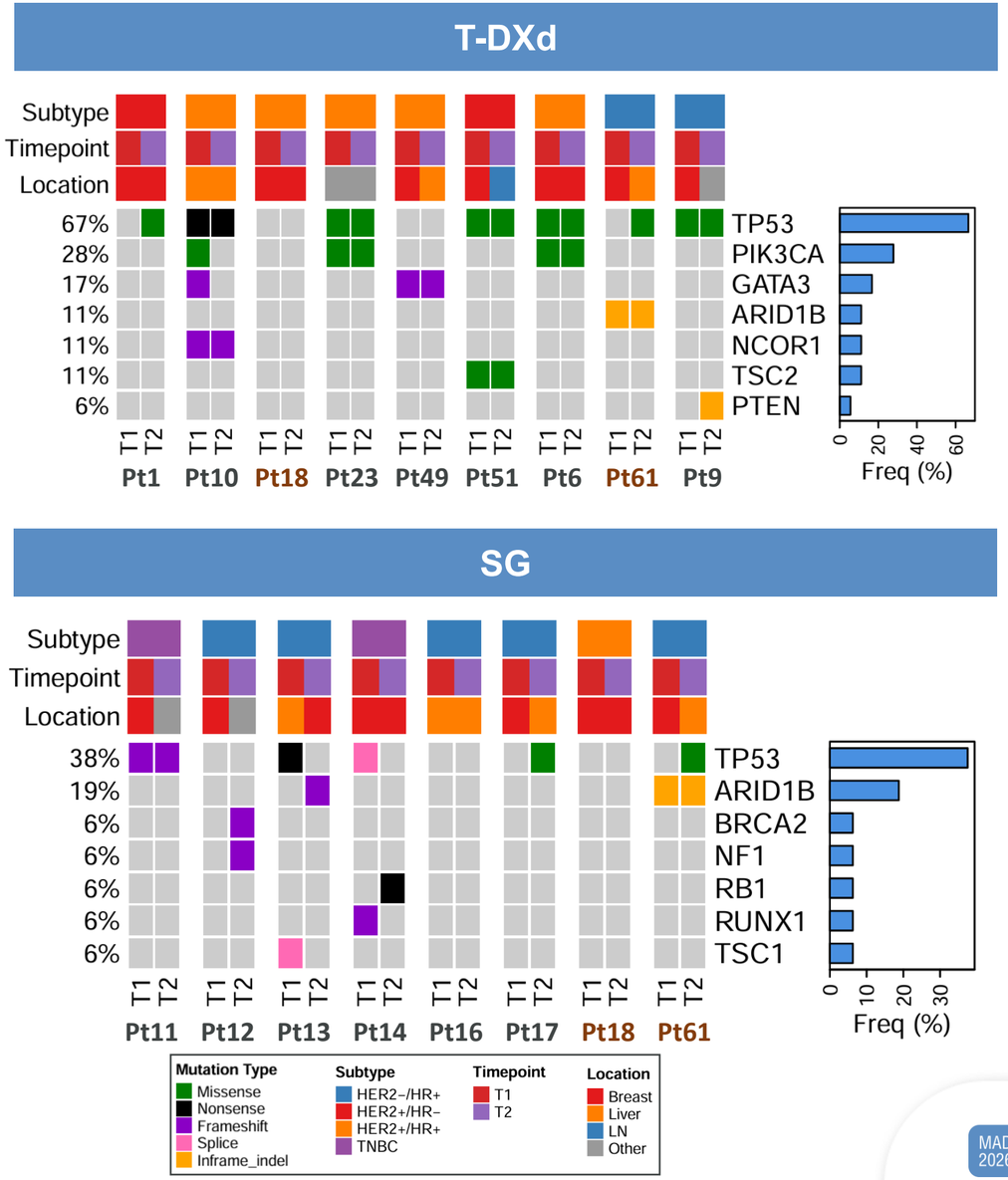
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IHC, immunohistochemistry; MS, mass spectrometry.

TP53 Mutations are Acquired in a Subset of Tumors at ADC Progression



- **Acquired *TP53* mutations** were detected after **T-DXd** and/or **SG** (**3/15 patients, 20%**)
- These mutations included two hotspot mutations and one loss-of-function alteration



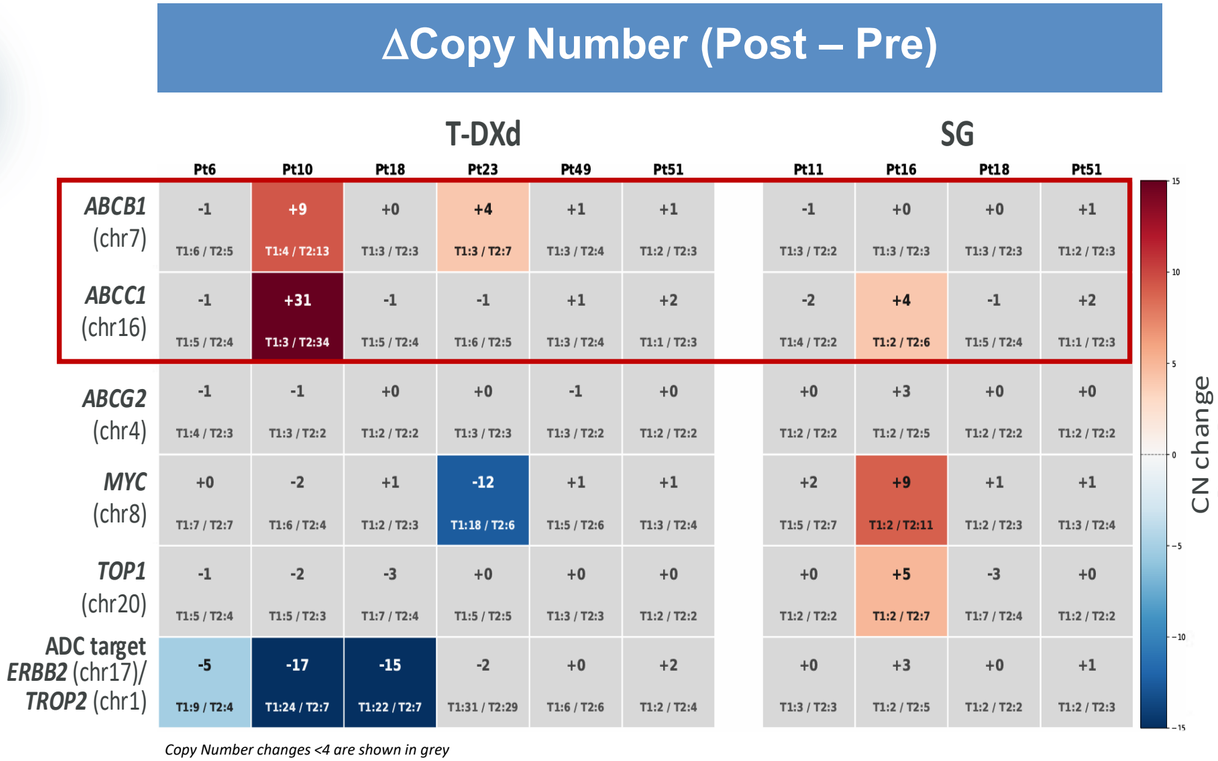
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Acquired ABCB1/ABCC1 Copy-Number Gains Emerge at ADC Progression

- ***ABCB1*** and ***ABCC1*** showed **acquired copy-number gains at ADC progression in 3/8 (37.5%)**, supporting drug efflux as a potential resistance mechanism.
- One tumor exhibited **marked co-amplification of both efflux pumps** (*ABCB1*: 4⟶13; *ABCC1*: 3⟶34).





Copy Number changes <4 are shown in grey

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Proteomic Changes Support Multiple Co-Existing Mechanisms of T-DXd Resistance

- **ABCB1** and **ABCC1** protein expression **increased in 5/7 (71%) and 4/7 (57%) T-DXd-treated tumors**, reinforcing the NGS findings.

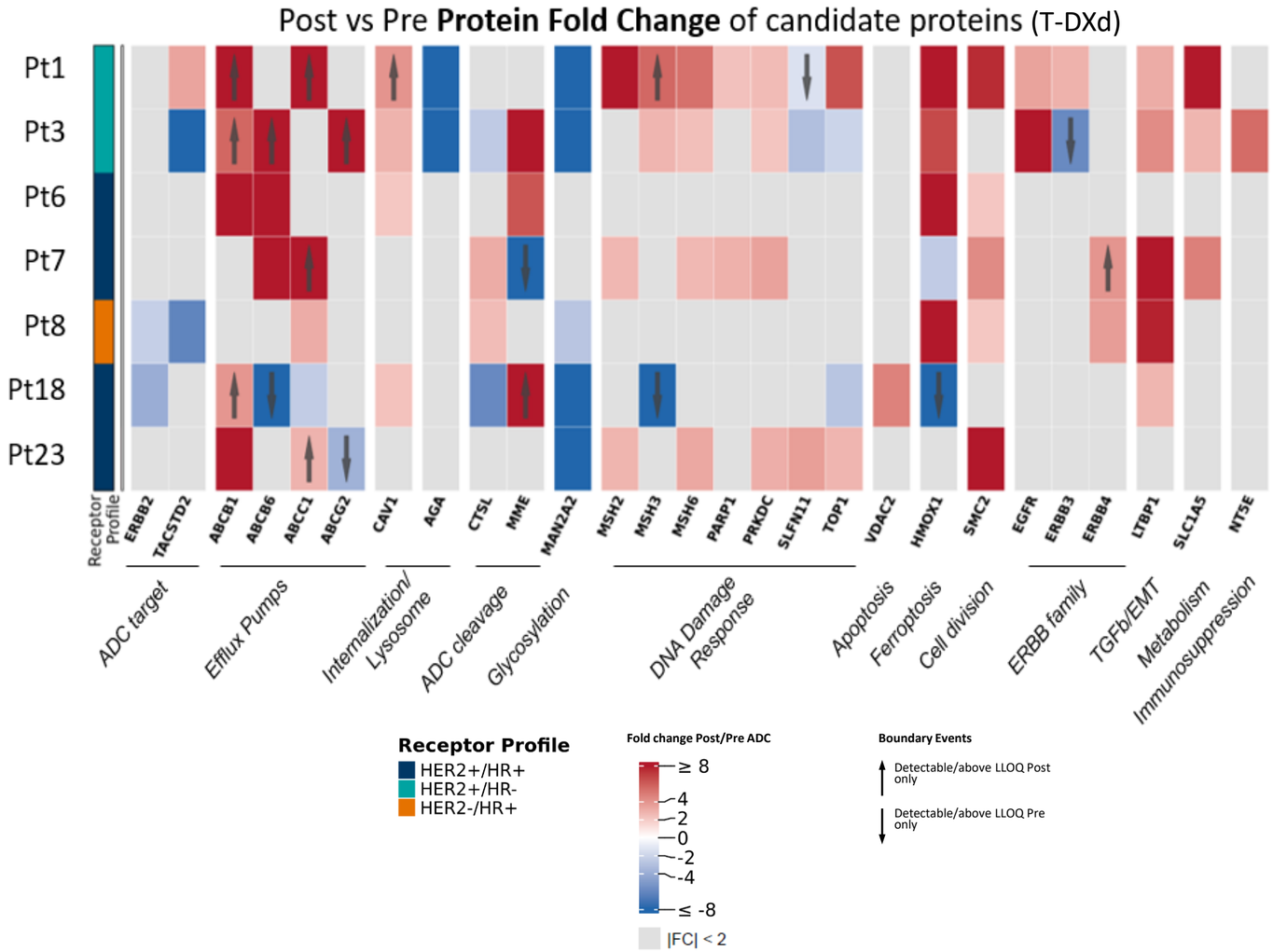


- **MAN2A2 expression decreased in 5/7 (71%) tumors,** suggesting altered glycosylation as an additional potential resistance mechanism.



- Recurrent changes in **DNA damage response** and **ferroptosis** markers were also observed.

By contrast, no recurrent ADC-related proteomic change was observed after **SG**.



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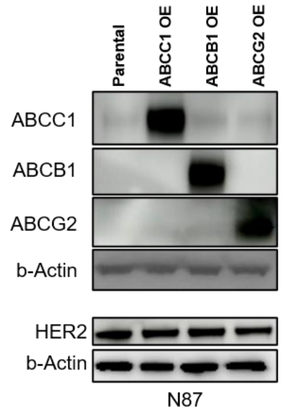
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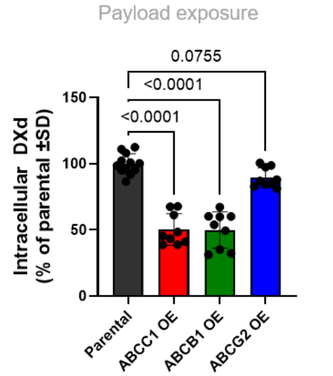
Preclinical Models Validate Efflux and Impaired Internalization as Mechanisms of T-DXd Resistance

- **ABCB1 or ABCC1 overexpression** in NCI-N87 cells significantly **reduced intracellular payload exposure** and T-DXd sensitivity.

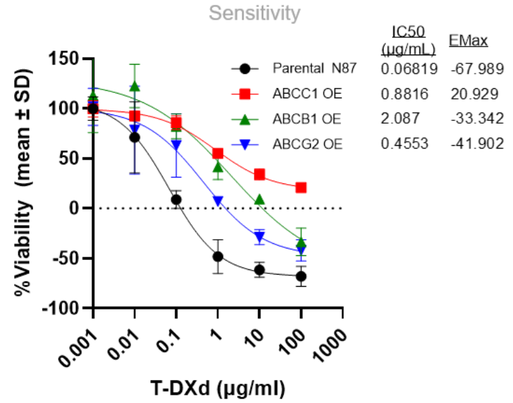
- siRNA-mediated **MAN2A2 silencing** in KPL4 cells **decreased T-DXd binding and internalization**, reducing intracellular payload exposure and T-DXd sensitivity.

Efflux pump overexpression impacts payload exposure *in vitro*



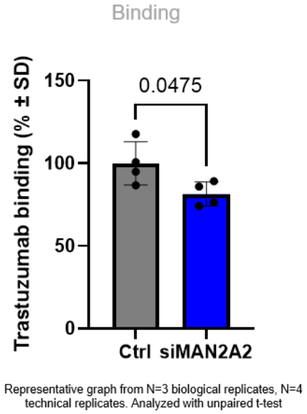


N=3 biological replicates with N=4 technical replicates
ANOVA with Dunnett's post-hoc

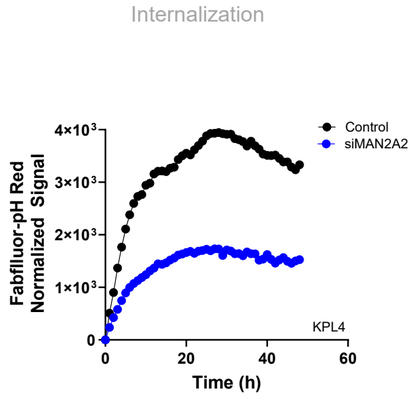


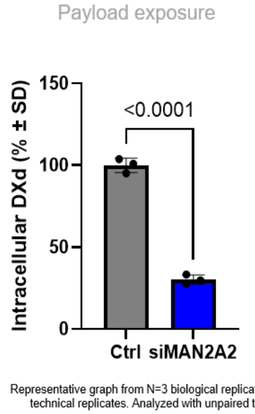
Representative curves from N=3 biological replicates, N=4 technical replicates

MAN2A2 downregulation reduces T-DXd internalization *in vitro*

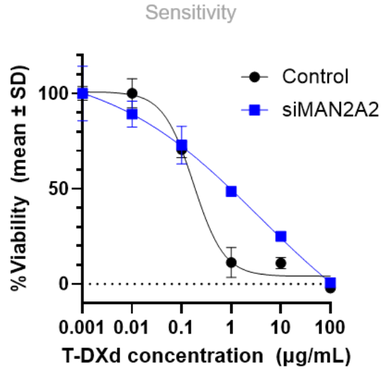


Representative graph from N=3 biological replicates, N=4 technical replicates. Analyzed with unpaired t-test





Representative graph from N=3 biological replicates, N=3 technical replicates. Analyzed with unpaired t-test



Representative curves from N=3 biological replicates, N=2 technical replicates. Non-targeting siRNA as control (Ctrl)

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Conclusions

- **ADC resistance is multifactorial**, with complete target loss being uncommon and multiple resistance mechanisms co-existing within individual tumors.
- Functional studies implicate **ABCB1/ABCC1-mediated drug efflux** and **MAN2A2-associated internalization defects** as mechanisms of T-DXd resistance
- **Payload-related mechanisms** may be critical determinants of ADC resistance, highlighting the limitations of relying on target expression alone.



LIMITATIONS: These results are hypothesis-generating, warranting confirmation in larger, prospective studies.

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THANK YOU

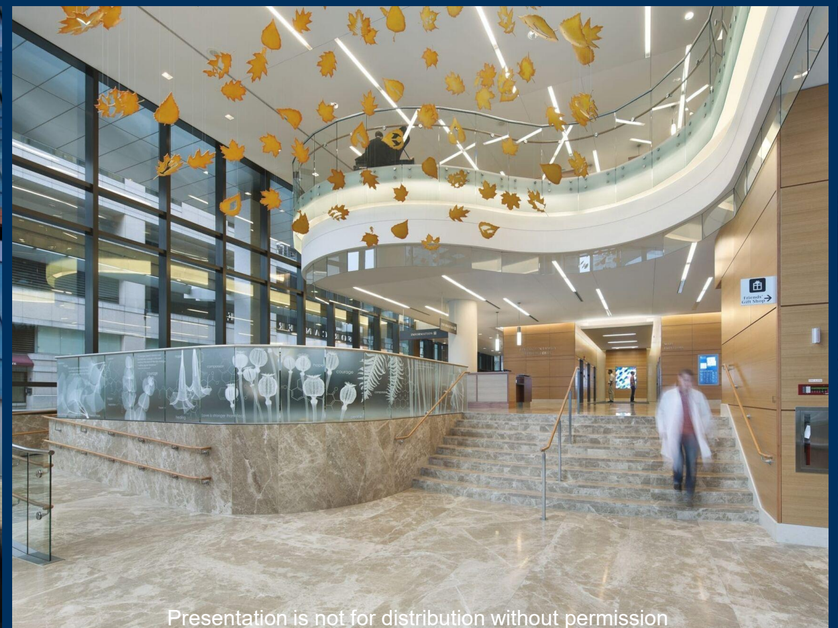
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