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Development and validation of a 19-feature classifier that predicts response to neoadjuvant trastuzumab emtansine (T-DM1) in breast cancer patients

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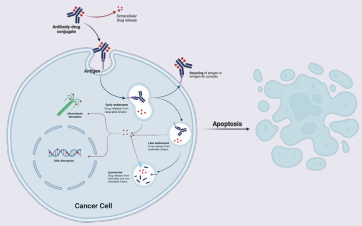
Background

- Antibody drug conjugates (ADCs) have complex mechanisms of action involving ADC intrinsic factors (antibody, linker, payload) as well as cellular processes necessary for processing ADCs (endocytosis, lysosome, resistance)[1].

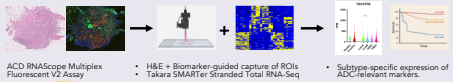


- Current diagnostic assays such as IHC fail to capture this complexity and are insufficient for patient stratification[2-4].
- **Objective** – To address these limitations, we determined the subtype-specific expression of ADC-relevant markers and their association with survival in a retrospective cohort of 1,082 breast cancer patients. Then we developed and validated a predictive model consisting of a tailored panel of ADC-markers to predict response to T-DM1 using data from the phase 2, prospective I-SPY2 trial.

References: **1.** Dumontet C, et al. Nature Reviews Drug Discovery 22, 641–661 (2023); **2.** Modi, S. et al. N Engl J Med. 387, 9–20 (2022); **3.** Bardia, A. et al. N Engl J Med. 384, 1529–1541 (2021); **4.** Bardia, A. et al. Annals of Oncology 32, 1148–1156 (2021); **5.** Paul, E.D. et. al. medRxiv 2023.12.05.23299341; doi: https://doi.org/10.1101/2023.12.05.23299341 (2023).

Methods

The mFISHseq (Multiplex8+) assay[5]



- ACD RNAScope Multiplex Fluorescent V2 Assay
  - Estrogen (*ESR1*)
  - Progesterone (*PGR*)
  - Her2 (*ERBB2*)
  - Ki67 (*MKI67*)
- H&E + Biomarker-guided capture of ROIs
- Takara SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian
- NovaSeq 6000 –100M reads/sample (2 x 100 PE)
- Subtype-specific expression of ADC-relevant markers.
- Multivariate Cox models for association with survival.

Retrospective cohort

- 1,082 FFPE breast tissues
- Clinicopathological data
  - IHC and 18 years follow up
- Ethics Committee of the Bratislava Self-Governing Region (Ref. No. 05320/2020/HF)
- Ethics Commission of the Medical University of Graz on behalf of Biobank Graz (No. 34-354 ex 21/21, 1158-2022)

I-SPY2 trial (NCT01042379)

- T-DM1 arm: 52 HER2+ patients (35 ER+ and 17 ER−; 30 patients achieving pCR).
- Paclitaxel+Trastuzumab arm: 31 HER2+ patients (19 ER and 12 ER−; 8 patients achieving pCR).
- Paclitaxel, Pertuzumab, Trastuzumab arm: 44 HER2+ patients (29 ER+ and 15 ER−; 26 patients achieving pCR).
- Logistic modeling w/elastic net (10-fold CV).

Results – ADC marker expression and association with survival

1 Top ADC targets show high inter-individual variability and subtype-specific expression patterns.

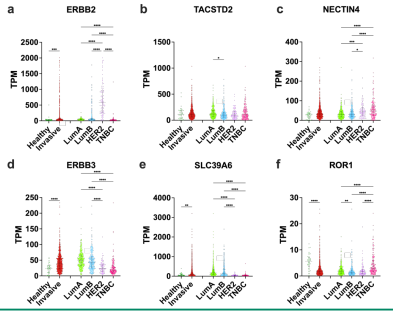


Figure 1. Expression of ADC targets in healthy versus invasive breast cancer tissue. Expression of ADC antigen targets assessed by RNA sequencing in healthy tissue (n=38), invasive breast cancer tissue (n=1,013), and Luminal A (LumA, n=432), Luminal B (LumB, n=313), HER2+ (n=87), and triple negative breast cancer (TNBC, n=181) IHC-surrogate subtypes. *p < 0.05, **p < 0.01 ***p < 0.001, ****p < 0.0001.

2 ADC processing-related genes are associated with overall survival in multivariate Cox proportional hazards models in a subtype-dependent manner and occasionally in opposing directions. These prognostic signatures could inform patient stratification for ADC treatment.

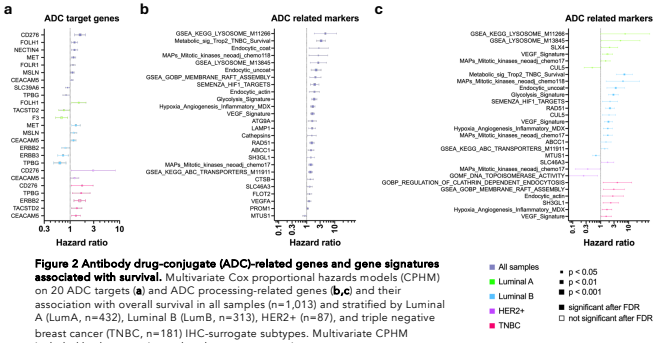


Figure 2 Antibody drug-conjugate (ADC)-related genes and gene signatures associated with survival. Multivariate Cox proportional hazards models (CPHM) on 20 ADC targets (**a**) and ADC processing-related genes (**b,c**) and their association with overall survival in all samples (n=1,013) and stratified by Luminal A (LumA, n=432), Luminal B (LumB, n=313), HER2+ (n=87), and triple negative breast cancer (TNBC, n=181) IHC-surrogate subtypes. Multivariate CPHM included both tumor size and node status as covariates.

Results – Development and validation of T-DM1 predictor

3 ADC-processing genes and gene signatures are associated with pathological complete response in the T-DM1 arm of the I-SPY2 trial.

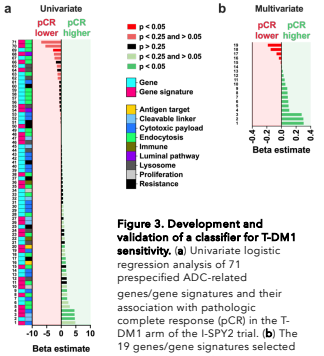


Figure 3. Development and validation of a classifier for T-DM1 sensitivity. (**a**) Univariate logistic regression analysis of 71 prespecified ADC-related genes/gene signatures and their association with pathologic complete response (pCR) in the T-DM1 arm of the I-SPY2 trial. (**b**) The 19 genes/gene signatures selected in the multivariate logistic regression and their association with pCR. Green bars associated with pCR; red bars associated with no pCR; black bars no association..

4 A 19-feature classifier containing features tailored to T-DM1 (ERBB2), endocytosis, lysosome function, and microtubule targets of the maytansine payload outperformed both HER2 IHC 42.3% of patients (n=22/52, 42.3% of patients w/no pCR) and *ERBB2* mRNA in predicted pCR to T-DM1.

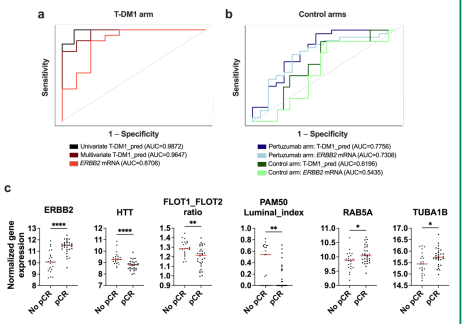


Figure 4. Development and validation of a classifier for T-DM1 sensitivity. ROC curves showing performance of T-DM1_pred classifiers relative to *ERBB2* mRNA alone in the T-DM1 arm (**a**) and in both the pertuzumab and taxane/anthracycline control arms (**b**). Univariate T-DM1_pred is a single score derived from all 19 features, while multivariate T-DM1_pred includes all 19 features in a multivariate regression model. AUC = area under the curve. (**c**) Scatter plots showing the distribution of selected genes/gene signature scores of the T-DM1_pred classifier in patient samples according to pCR.

Summary and Conclusions

- Using mFISHseq (also known as Multiplex8+[5]), we characterized the cellular pathways involved in ADC mechanisms of action, revealing high inter-individual variability, unique subtype-specific expression patterns, and prognostic groups that may be relevant for stratifying patients into ADC-responsive subgroups.
- As a proof-of-concept, our T-DM1 predictor comprised of 19 genes/gene signatures tailored to the specific mechanism of action of T-DM1 showed superior predictive performance compared to HER2 IHC and *ERBB2* mRNA.
- These data create a foundation and roadmap for ADC patient selection by tailoring gene signatures to key features of the ADC: antigen and payload target (topoisomerase, microtubule, or DNA), cleavable (enzyme or acid labile) or noncleavable (lysosome processing) linker, and mechanisms of resistance (ABC transporters, glucuronidation enzymes).

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