Beyond bulk: Resolving RNASeq/Mass Spectrometry/IHC discrepancies with multiplexed spatial profiling and 3D cluster analysis to refine HER3 (bs)Ab and (bs)ADC therapeutic strategies

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AACR 2026 | Presentation number **5738**

SUMMARY

Assessment of target expression for mono- and bi-specific antibodies (bs)Abs and antibody-drug conjugates (bs)ADCs can be challenging due to variable correlation between bulk RNASeq, proteomics and immunohistochemistry (IHC) data. While IHC is sufficient to address single target expression levels at single cell level, multiplexed proteomics is required to assess spatial distribution and co-expression of multiple targets for bsAb and bsADC use.

To address these challenges, we performed an in-depth characterization of HER3 and an undisclosed target (Protein X) across multiple omics platforms in patient-derived xenografts (PDXs) for a bsADC targeting HER3 and Protein X.

HER3 mRNA indeed correlates only moderately with Mass Spectrometry-derived (MS) HER3 protein intensities (Figure A). Similarly, HER3 protein intensities do not properly reflect IHC-derived HER3 protein levels (Figure B) besides not capturing the details of HER3 heterogeneity as IHC does (Figure C). Notably, none of these analytes sufficiently predicts response of in vivo PDXs (S=sensitive, R=resistant) to the HER3/Protein X bsADC.

Finally, the proportion of HER3- and/or Protein X-positive cells was assessed by multiplexed proteomics to investigate the co-expression pattern of both targets and their effect on response to HER3/Protein X bsADC treatment in vivo (Figure D, E). While co-expression by itself was non-predictive, the 3D multifactorial combination of HER3 and Protein X expression levels, together with the proportion of co-expressing cells, show a tendency towards HER3/Protein X bsADC resistance being associated with higher H-scores of both targets (Figure F). However, another PDX model with similar features (#2-S) showed sensitivity to the same treatment.

A larger cohort of PDX models with a larger target expression range needs to be analysed to build more reliable associations between target expression and in vivo response.

METHODS

PDX in vivo studies conducted with Crown Bioscience Inc.

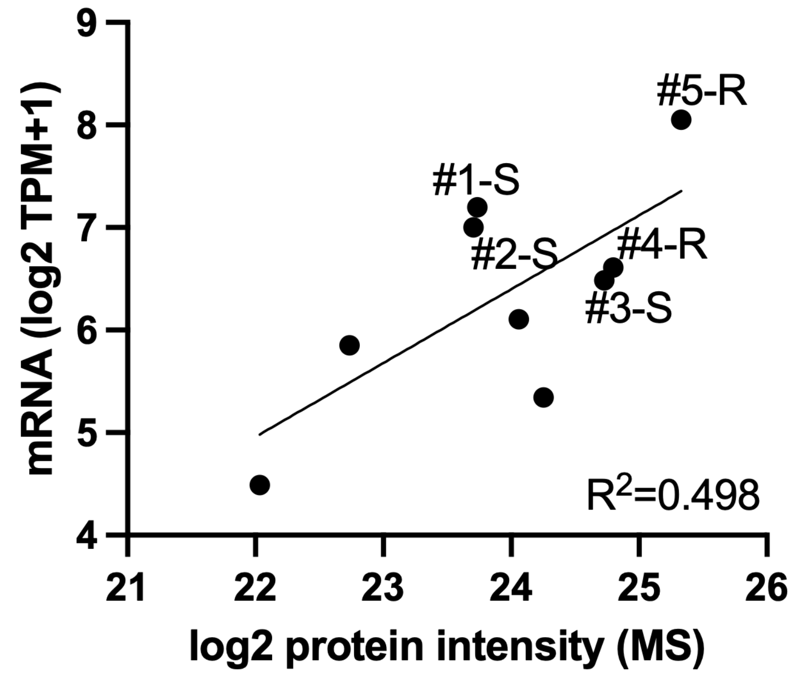
HER3 RNASeq and MS proteomics data provided by Crown Bioscience, Inc. as part of an Omics Data License Agreement.

Multiplexed proteomics scanned on the Akoya PhenoImager HT Image system and analyzed on the HALO Image Analysis platform.

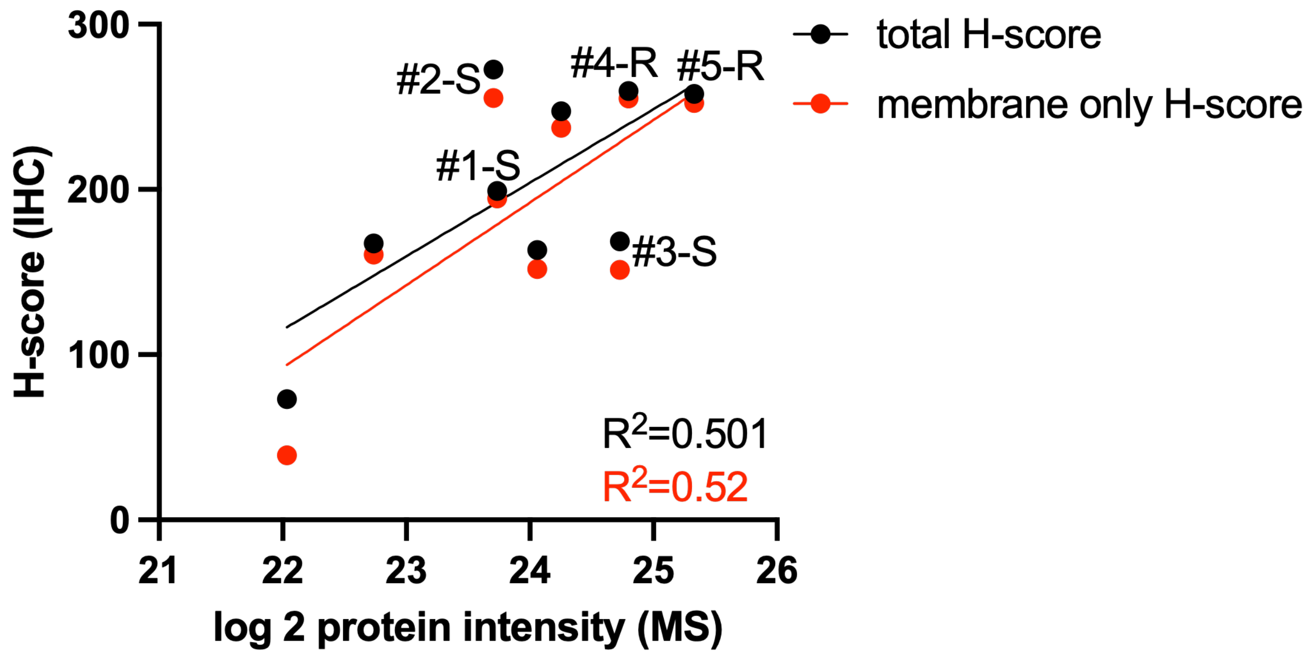
REFERENCES

[1]A. Attinger et al., Enhancing therapeutic efficacy and overcoming resistance with a novel dual payload antibody-drug conjugate Technology, Poster #1683, AACR 2026

RESULTS

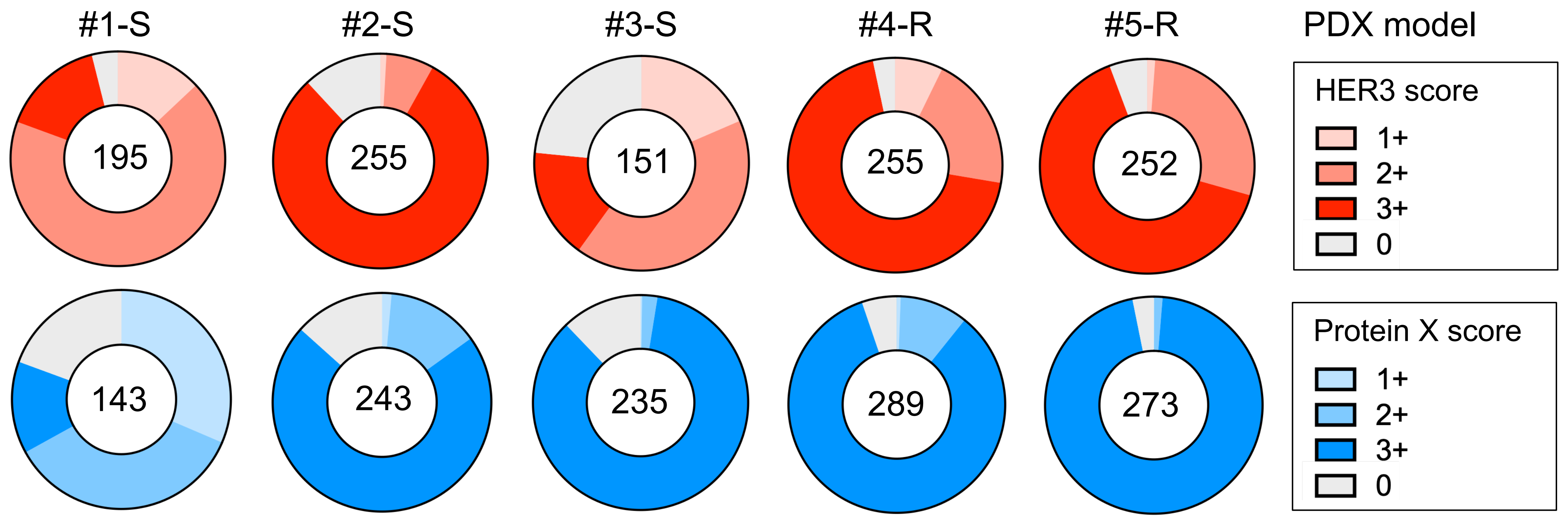


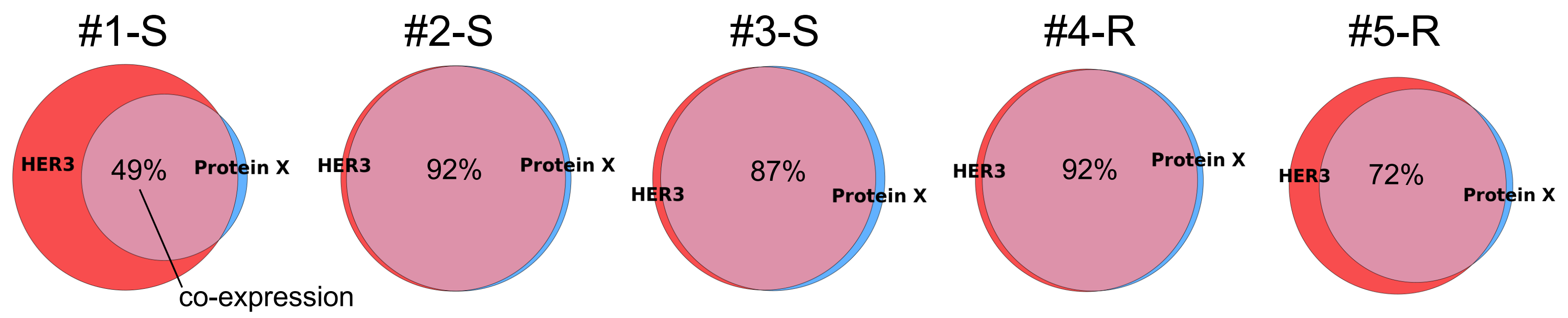
▲**Figure A**: Linear correlation of HER3 mRNA expression levels and HER3 protein intensities in PDX models. R=resistant, S=sensitive to HER3/Protein X bsADC



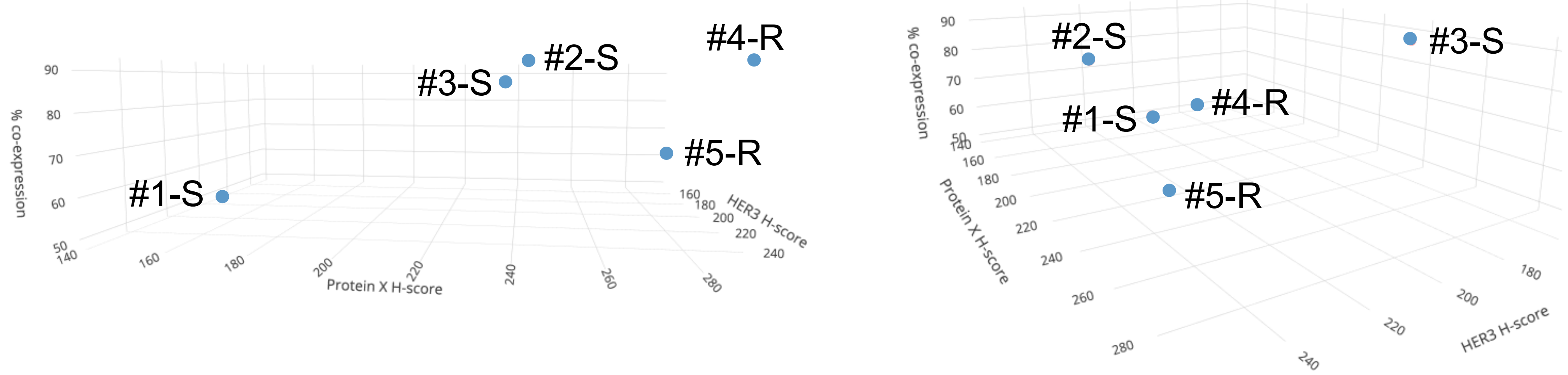
▲**Figure B**: Linear correlation of total and membrane-only HER3 H-scores (IHC) and HER3 protein intensities in PDX models. R=resistant, S=sensitive to HER3/Protein X bsADC

▼**Figure C**: Detailed HER3 and Protein X IHC results (membrane only) from PDX models display tumor heterogeneity. Distribution of staining intensities: absence of staining (0), weak (1+), moderate (2+) and strong (3+) staining. H-scores in the center of the circles. R=resistant, S=sensitive to HER3/Protein X bsADC

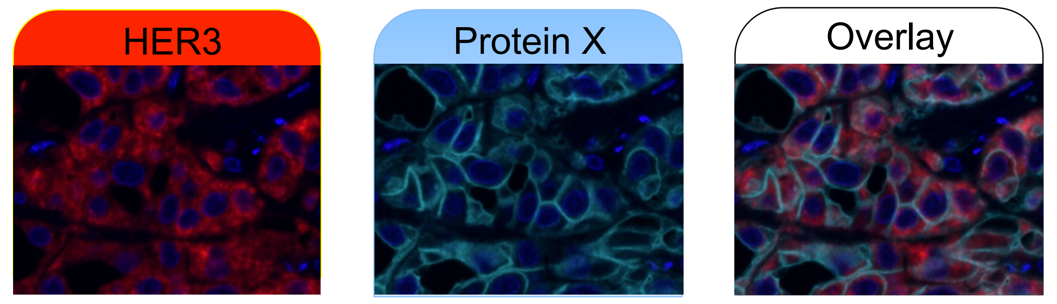




▲**Figure D**: Multiplexed Proteomics. Venn diagrams with quantifications (%) of HER3 and Protein X co-expressing cells in PDX models (created with eulerr). All PDX models have a high percentage of cells with target co-expression and a very low percentage of Protein X-positive/HER3-negative cells. The percentage of HER3-positive/Protein X-negative cells varies across models with highest prevalence in PDX model #1-S (45%) and #5-R (21%)



▲**Figure F**: 3D multifactorial scatter plot combining HER3 and Protein X IHC-derived H-scores (Figure C) and % of HER3/Protein X co-expressing cells from multiplexed proteomics (Figure D).



◂**Figure E**: Multiplexed Proteomics. Example of immunofluorescent staining of HER3 (red), Protein X (turquoise) and nuclei (DAPI, dark blue) in PDX #3-S

CONCLUSION

The moderate correlation between HER3 mRNA and protein levels suggests the use of a protein-based HER3 analyte for the selection of PDX models to test HER3/Protein X bsADC in vivo efficacy. However, several protein-based assessments fail to predict in vivo response. Neither bulk MS nor IHC - which additionally captures complexities of tumor heterogeneity and staining intensity distributions - correlate with sensitivity. Even the quantified proportions of single-positive and HER3/Protein X co-expressing tumor cells, as quantified by multiplexed proteomics, are not predictive. Finally, the multifactorial combination of H-scores and target co-expression suggests a tendency toward HER3/Protein X bsADC resistance in PDX models with higher target expression. This observation warrants further evaluation in a larger PDX cohort with a higher dynamic range of HER3 and Protein X expression levels. Additionally, target-independent mechanisms such as internalization rate, linker stability and payload sensitivity/combinations[1] must be accounted for and investigated.

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