Quantitative measurement of HER2 and TROP2 predicts outcomes with trastuzumab deruxtecan in metastatic breast cancer

C. Jack Robbins[1], Wei Wei[1], Nay N.N. Chan[1], Mengni He[1], Julia Benanto[1], Victoria H. Fan[3], Deborah Doroshow[4], Ian Krop[2], David L. Rimm[1,2]

[1]Department of Pathology, Yale School of Medicine; [2]Department of Medicine (Oncology), Yale School of Medicine; [3]Department of Neuroscience, Yale School of Medicine; [4]Department of Medicine (Oncology), Ichan School of Medicine at Mount Sinai

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Summary

Background

- ADCs targeting HER2 (trastuzumab deruxtecan, T-DXd) and TROP2 expand options in mBC, but optimal patient selection is unclear
- **Aim**: Evaluate association between HER2/TROP2 Troplex™ measurements and time-on-treatment (ToT) with T-DXd.

Methods

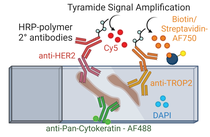
Retrospective Yale cohort of 65 mBC patients treated with T-DXd (4/2020–2/2025) → FFPE samples stained for HER2, TROP2, CK (Troplex). Analyzed in QuPath with Qymia. HER2/TROP2 quantified (amol/mm²) → ToT defined from T-DXd start to progression or discontinuation for any reason (≤ 2 years) → Associations with ToT evaluated by median split (KM/log-rank) and Cox models

Cohort Summary and Results

- Breast cancer subtypes: 19 HER2+, 35 HER2–/HR+, 11 TNBC
  - 78.5% metastatic biopsies
  - Median 18.4 months from sampling to T-DXd
- Median biomarker expression:
  - HER2 3,554 amol/mm² (range 15–8,232)
  - TROP2 2,868 amol/mm² (range 0–23,383)
- Above-median HER2: longer ToT (12.8 vs 7.5 mo; HR=0.51; p=0.019)
- Above-median TROP2: shorter ToT (5.5 vs 12.8 mo; HR=1.74; p=0.050)
- Each +2,000 amol/mm² TROP2 associated with shorter ToT (HR=1.17; p=0.001)
  - Negative association of TROP2 expression persisted in non-TNBC and non–HER2-amplified subsets

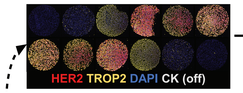
Troplex™: Quantitative HER2 & TROP2 immunofluorescence assay

Immunofluorescence Assay

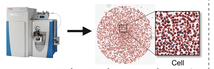


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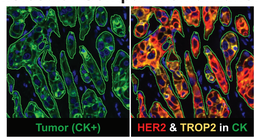
Cell Line Array Standard



HER2 & TROP2 Quantification:
LC-MS/MS (protein per cell)

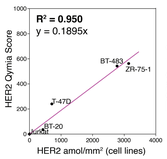


Molecular Compartmentalization



Qymia Score ~ $\frac{\Sigma\ Intensity}{\Sigma\ Compartment\ Area\ (\mu m^2)}$

Convert Qymia Score to $\frac{amol}{mm^2}$



Report Measurements

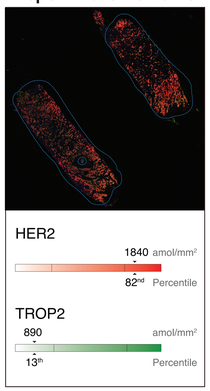


Figure 1: Overview of Troplex™ assay. Published in Robbins et al., *JCO Precis Oncol.* 2025. Qymia available at https://github.com/snibbor/Qymia-in-QuPath

Yale T-DXd Treated Retrospective Cohort

Cohort Summary

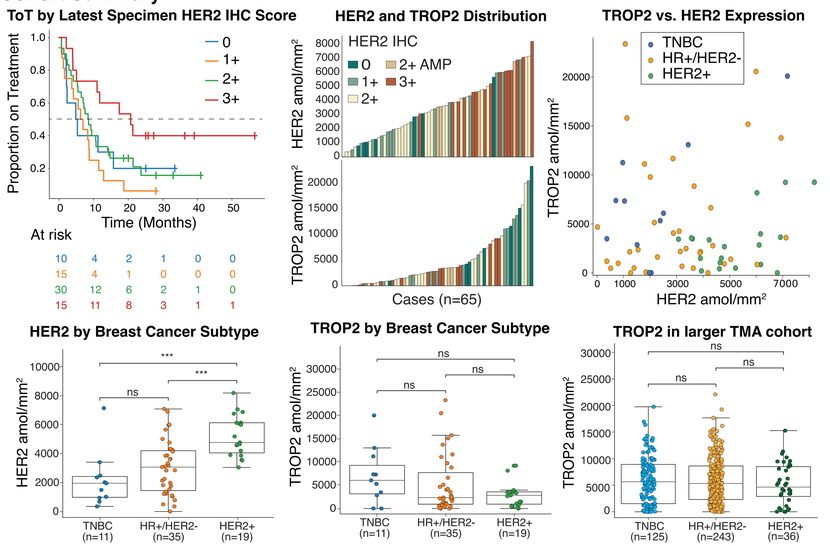


Figure 2: Cohort summary for 65 Yale mBC patients treated with T-DXd showing real-world PFS by latest HER2 IHC score, quantitative HER2 and TROP2 distributions, and their relationship across cases. Higher HER2 IHC scores correspond to higher continuous HER2 QIF levels and numerically longer ToT, whereas TROP2 is broadly expressed with no correlation to HER2. Box-and-whisker plots compare HER2 and TROP2 levels across TNBC, HR+/HER2-, and HER2+ subtypes in the T-DXd cohort. TROP2 distributions are similar to a larger independent Yale breast cancer TMA cohort.

Trastuzumab Deruxtecan Time-on-Treatment with HER2 and TROP2 Expression

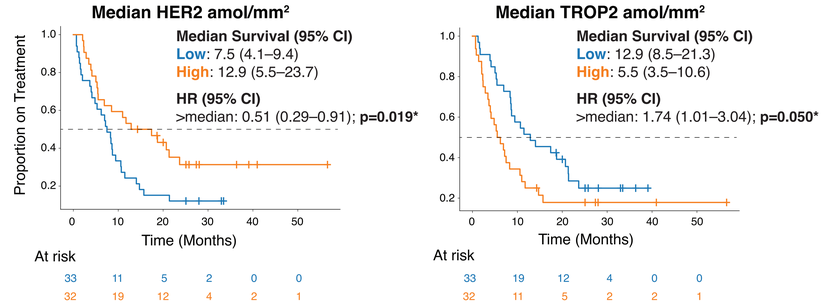
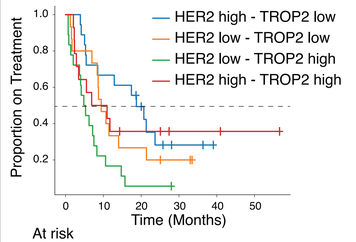


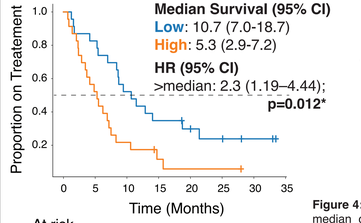
Figure 3: Time on T-DXd by quantitative HER2 and TROP2 expression. Patients with HER2 above the cohort median had longer ToT than those below the median (12.9 vs 7.5 months; HR 0.51, p=0.019), whereas higher TROP2 expression was associated with shorter ToT (5.5 vs 12.9 months; HR 1.74, p=0.050).

HER2 & TROP2 Stratification by Troplex™ assay

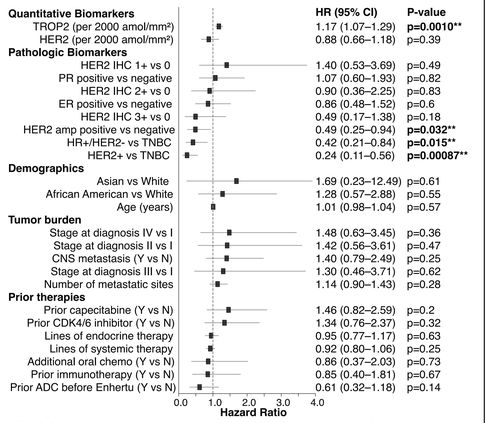
Troplex HER2 & TROP2, median-cutpoints



HER2-low only ToT by TROP2 amol/mm²



T-DXd ToT Univariate Hazard Ratios



TROP2 expression remains a negative predictor within subgroups

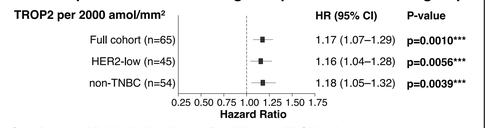


Figure 4: Stratification of T-DXd ToT by Troplex™ HER2 and TROP2. Kaplan–Meier curves using median cutpoints show patients with HER2-high/TROP2-low have the longest ToT, whereas TROP2-high groups, particularly HER2-low/TROP2-high, have the poorest outcomes. Forest plots from univariate CoxPH models demonstrate that higher TROP2 (per 2000 amol/mm²) is associated with shorter ToT and remains a significant negative predictor across cohort subgroups.

Conclusions & Future Directions

- Quantitative HER2 by Troplex™ correlates with longer T-DXd time-on-treatment
- Higher TROP2 is unexpectedly associated with shorter T-DXd benefit
- **Findings are hypothesis-generating and require validation in larger, independent cohorts**
  - 86 additional cases for Yale Real World T-DXd Retrospective Study
- **Can HER2 & TROP2 amol/mm² help select between T-DXd or SG? Define thresholds for response.**
  - Ongoing clinical studies using Troplex assay:
    - QuantifyHER (TBCRC), RELIEVE (DFCI), TRIO B12-TALENT (US-TRIO), HER2 Paradigm

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