QuantifyHER (Trial In Progress): Quantitative Immunofluorescence And/Or Messenger RNA To Measure HER2 Expression In Patients With Metastatic Breast Cancer Treated With Trastuzumab Deruxtecan (Translational Breast Cancer Research Consortium Trial #066)

Eleanor Taranto[1], Fawn White[1], Desirae Chandran[1], Emma Walinsky[1], Shannon Deluca[1], Lauren Bayne, E. Paul Wileyto[2], Anupama Shirali[3], Charles J. Robbins[3], Yara Abdou[4], Jesus Anampa[5], Marija Balic[6], Jenna V. Canzoniero[7], Natalie C. Chen[8], Frederick Howard[9], Laura Huppert[10], Adriana Kahn[11], Candace Mainor[12], Jason A. Mouabbi[13], Vidhya Nair[14], Traci DeGeer[15], Jodi Weidler[16], Thomas P. McNicholas[15], Christine Serway[17], Joachim Reischl[18], Michael Bates[16], Robert Monroe[17], David L. Rimm[3], Angela DeMichele[1]

on behalf of the Translational Breast Cancer Research Consortium

[1]Division of Hematology/Oncology, University of Pennsylvania, Philadelphia, PA, [2]Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA, [3]Department of Pathology, Yale School of Medicine, New Haven, CT, [4]Division of Oncology, University of North Carolina School of Medicine, Chapel Hill, NC, [5]Department of Oncology, Montefiore Einstein Comprehensive Cancer Center, New York, NY, [6]Division of Oncology, Hillmans Cancer Center, University of Pittsburgh, Pittsburgh, PA, [7]Division of Oncology, Johns Hopkins Sidney Kimmel Cancer Center, Baltimore, MD, [8]Department of Medicine, Section of Hematology and Oncology, Baylor College of Medicine, Houston, TX, [9]Biological Sciences Division, Section of Hematology/Oncology, University of Chicago, Chicago, IL, [10]Department of Medicine, University of California San Francisco, San Francisco, CA, [11]Department of Medicine, Division of Medical Oncology and Hematology, Yale School of Medicine, New Haven, CT, [12]Department of Internal Medicine, Division of Hematology/Oncology, Georgetown University School of Medicine, Washington, DC, [13]Department of Breast Medical Oncology, UT MD Anderson Cancer Center, Houston, TX, [14]Division of Hematology/Oncology, Fred Hutch/University of Washington School of Medicine, Seattle, WA, [15]Open Innovation, Leica Biosystems, Richmond, IL, [16]Medical/Scientific Affairs and Strategy, Oncology, Cepheid, Sunnyvale, CA, [17]Oncology, Danaher Diagnostics, Washington, DC, [18]External Innovation Diagnostics, Danaher Diagnostics, Washington, DC.

Background

- Antibody drug conjugates (ADCs) can be effective treatment for patients (pts) with metastatic breast cancer (MBC), but they can carry unique toxicities (pneumonitis, stomatitis, etc.).
- Trastuzumab Deruxtecan (T-DXd) is a HER2-targeted ADC, and is approved for pts with MBC expressing low/ultra-low levels of HER2 by Immunohistochemistry (a.k.a. IHC 1+, IHC 2+ FISH negative, or IHC 0+).
- It is unclear who will benefit from T-DXd, and whether or how HER2 expression predicts response or duration of response.
- This is in large part because of the poor reliability of HER2 IHC scoring at the low end of the spectrum.
- Significant inter-reader variability exists in the assessment of HER2 by IHC (See **Figure 1**).

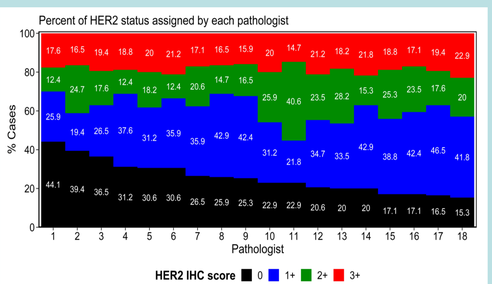


Figure 1: Discordance across 18 pathologists reading 170 cases at 15 institutions. The proportion of IHC 0 cases ranged from >40% to <20% that were called IHC 0. *Figure taken from Robbins et al., Mod Path 2023*

Contact: For further information about this project, please contact eleanor.taranto@pennmedicine.upenn.edu

Quantitative Assessment Methods

Quantitative Immunofluorescence (QIF): TROPLEX assay, measuring HER2 and TROP-2 proteins simultaneously on archival tissue

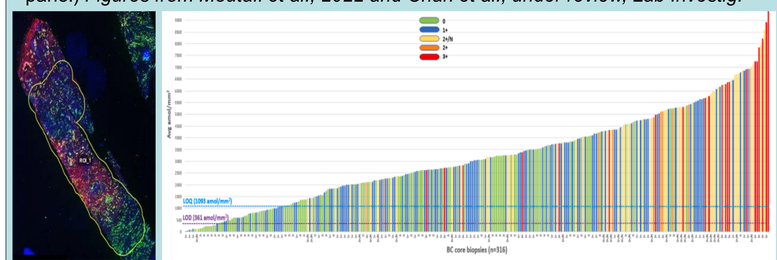
- Analytic validation cohort of >400 retrospective samples with ~50% of HER2 IHC 0 cases with HER2 QIF > limit of quantification (LOQ)
- Relationship demonstrated between QIF and time-to-next-treatment (TTNT) in small, retrospective cohort of patients treated with T-DXd (*Tarantino et al., ESMO 2024)*

Messenger RNA Assessment (mRNA): Cepheid's Xpert® Breast Cancer STRAT4 (RUO version)† cartridge-based mRNA assay on archival tissue

- <2 hours to run, with *ESR1, PGR, ERBB2,* and *MKi67* outputs. High concordance in retrospective data between STRAT4 *ERBB2* mRNA and HER2 IHC/ISH, with greater potential range

†For Research Use Only. Not for use in diagnostic procedures.
(Outside of the U.S., STRAT4 is a CE-IVD in vitro diagnostic medical device. Not available in all countries. Not available in the U.S.)

Figure 2: QIF performed on pathologist-selected range of interest using H&E (left panel) with a range of HER2 QIF (amol/mm2) above LOQ in most samples (right panel) *Figures from Moutafi et al., 2022 and Chan et al., under review, Lab Investig.*



Acknowledgements

• We are grateful to all the patients who generously volunteered to participate in this study.
• We thank the TBCRC investigators, research nurses, & study coordinators for their efforts on behalf of the patients.
• We are appreciative of the funding support provided to the TBCRC by its foundation partners:
  •The Breast Cancer Research Foundation
  •Susan G. Komen
•We are grateful for the support of Danaher/Cepheid in funding this study.

QuantifyHER Study Methods

QuantifyHER Study Schema

Eligibility:
- Metastatic breast cancer, histologically confirmed
- HER2 IHC<2+ (per local pathology)
- Measurable disease
- Available tissue for biomarker assessment
- Intention to initiate therapy with T-DXd

N=200 evaluable participants

Patient consent → Treatment with T-DXd per standard of care → Complete study participation at T-DXd discontinuation

Intravenous infusion once every 3 weeks (21-day cycle)

10 unstained FFPE slides (or tissue block) submitted to Rimm Lab (Yale) after enrollment → Analysis by TROPLEX QIF and STRAT4 mRNA assays → Endpoints: **Primary: Real-World ORR** Secondary: Real-World PFS

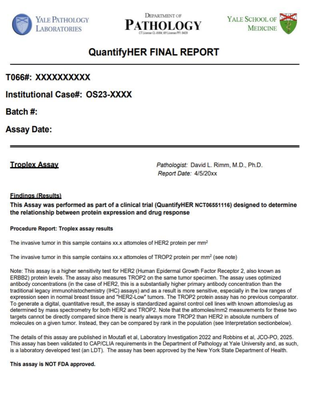
Submit most recent HER2 IHC <2+ biopsy that was the basis for T-DXd use, either:
1) Most recent HER2 <2+ metastatic biopsy (preferred)
OR
2) Most recent prior HER2 <2+ biopsy.
Bone samples accepted ONLY if no other sample is available

Return of Results

Pts can consent to receive results report

Report contains HER2/TROP2 QIF (as these are performed in CLIA validated setting)

Pts who opt in will receive report at the end of study participation (a.k.a. T-DXd discontinuation)


QuantifyHER FINAL REPORT

T066#: XXXXXXXXX
Institutional Case#: OS23-XXXX
Batch #:
Assay Date:

Troplex Assay

Pathologist: David L. Rimm, M.D., Ph.D.
Report Date: 4/5/20xx

Findings (Results)
This Assay was performed as part of a clinical trial (QuantifyHER NCT06551116) designed to determine the relationship between protein expression and drug response

Procedure Report: Troplex assay results

The invasive tumor in this sample contains xx.x attomoles of HER2 protein per mm²

The invasive tumor in this sample contains xx.x attomoles of TROP2 protein per mm² (see note)

Note: This assay is a higher sensitivity test for HER2 (Human Epidermal Growth Factor Receptor 2, also known as ERBB2) protein levels. The assay also measures TROP2 on the same tumor specimen. The assay uses optimized immunofluorescence chemistry (IFC) and digital pathology. The assay is more quantitative and sensitive than the traditional legacy immunohistochemistry (IHC) assays and as a result, is more sensitive, especially in the low ranges of expression seen in normal breast tissue and "HER2-Low" tumors. The TROP2 protein assay has no previous comparator. To generate a digital, quantitative result, the assay is standardized against control cell lines with known attomoles/µg as determined by mass spectrometry for both HER2 and TROP2. Note that the attomoles/mm2 measurements for these two targets cannot be directly compared since there is nearly always more TROP2 than HER2 in absolute numbers of molecules on a given tumor. Instead, they can be compared by rank in the population (see Interpretation section below).

The details of this assay are published in Moutafi et al, Laboratory Investigation 2022 and Robbins et al, JCO-PO, 2025. This assay has been validated to CAP/CLIA requirements in the Department of Pathology at Yale University and, as such, is a laboratory developed test (an LDT). The assay has been approved by the New York State Department of Health.

This assay is NOT FDA approved.

Participating Sites:

Penn (lead site)
Yale
Baylor
Georgetown
Johns Hopkins
MD Anderson
Montefiore
UNC
UPMC
UChicago
UCSF
Washington/Fred Hutch

(within the *Translational Breast Cancer Research Consortium (TBCRC)*)

Primary Objective:

To evaluate associations between **quantitative HER2** (QIF / mRNA) and **real-world response (rwORR)** to T-DXd in pts with HER2 IHC<2+ MBC starting T-DXd

Secondary Objectives:

- Evaluate associations between HER2 QIF/mRNA and real-world progression-free survival (rwPFS)
- Evaluate association between TROP2 QIF and rwORR and rwPFS to T-DXd
- Evaluate associations by ER expression
- Identify cutoff for HER2 QIF/mRNA, TROP2 QIF, or the combination, below which pts don't respond to T-DXd
- Determine whether combined mRNA + QIF measure is better than either tool alone at discriminating T-DXd response

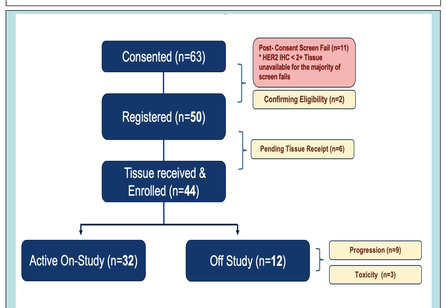


Figure 3: QuantifyHER Consort of pts consented and enrolled as of 11/13/2025.