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Sequencing full-length mRNA in whole blood of breast cancer patients for ADC therapy selection

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Background

- Identifying biomarkers for selection of antibody-drug conjugate (ADC) therapeutics is a critical, clinical unmet need. There are limited or only partially effective options for determining efficacy.
- T-DXd has demonstrated the value of granular quantification of HER2 expression for response prediction, but IHC/ISH testing has limited accuracy for HER2 low and ultralow patients.
- When determining ADC eligibility, the lack of reliable quantification can lead to under- or over-treatment. Developing an accurate and reliable quantification will enable safer and more effective ADC utilization.
- We have previously demonstrated[1] the effectiveness of a flexible long-read transcriptomics platform in characterizing HER2 expression.
- We further demonstrate this platform's ability to assess protein isoform variation in expressed HER2, with impacts on ADC response/resistance.
- We also show the application of whole blood biopsy to monitor healthy expression of ADC targets in blood to identify potential toxicity.

Methods and Data

- We have developed a novel platform that leverages long-read sequencing to capture full-length mRNA and generate isoform and sub-isoform level feature expression profiles.
- Specialized versions of this assay are suited for profiling solid tissue material, and for targeting full-length transcripts from blood[2].
- Using our solid tissue platform, we profiled 50 breast tumor samples with pathologist IHC categories HER2 0 (11), 1+ (21), 2+ (14), and 3+ (4).
- For 41 of these donors, we took whole blood samples at the time of tumor biopsy, which we profiled with our long-range assay.
- We also profiled 40 blood samples from donors confirmed breast cancer-free at the Western General Hospital, to provide a demographically comparable control population.
- We calculated per-gene/per-sample read counts and parts-per-million (PPMs) for 61,537 genes from the *Ensembl* reference[3].
- For a subset of genes serving as current candidate ADC targets, we used proprietary TAMA software to annotate and quantify isoform features including exons, introns, and combinations thereof.

Figure 1: Wobble Genomics' processing workflow applying long-read transcriptomics to solid/liquid biopsy samples.

Full-length transcripts give epitope resolution to HER2 expression

IHC quantifies a range of protein expression, including proteins not targeted by trastuzumab

- Trastuzumab, and by extension ADCs such as T-DM1 and T-DXd, target ECD subdomain IV, while standard HER2 IHC assays target the intracellular C-terminal tail[4] (aa 1244–1249; Fig. 2T).
- We measured HER2 expression based on all HER2 transcripts for 50 breast tumor tissue samples (see Methods), showing clear association with HER2 IHC classification ($p < 10^{-6}$; Fig. 3L).
- However, full-length transcripts showed widespread variation around the trastuzumab binding site (Fig. 2B).
- Alternately spliced products likely detected by IHC but untargeted by trastuzumab appeared in 92% of tumor and 68% of matched blood samples (prevalence of individual isoform features shown to right of Fig. 3L).
- These transcripts, detected by IHC but missed by trastuzumab, may act as sources of ADC resistance.

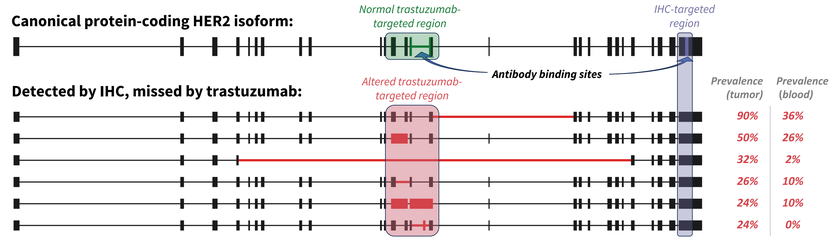

*Figure 2: HER2 locus on chromosome 17 (39.70-39.73Mb). **Top**: canonical HER2 isoform, with trastuzumab binding site and IHC binding site highlighted. **Bottom**: isoforms with alternate exon/intron splicing patterns in the trastuzumab-adjacent region.*

Quantifying trastuzumab-specific HER2 expression may identify HER2 low/ultralow responders

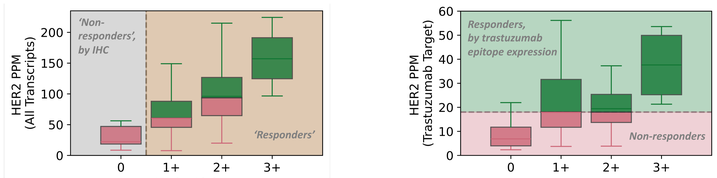

*Figure 3: Parts-per-million (PPM) HER2 reads, vs HER2 IHC score (n = 50). Shading within IHC classes represents proportions of responders/non-responders, as per DESTINY-Breast04/06. **Left:** Unfiltered PPM. **Right:** Epitope-filtered PPM.*

- DESTINY-Breast04/06[5] estimated response rates of ~80% (IHC 3+), ~57% (2+), ~49% (1+), and ~24*% (0).
- Applying these rates, we see many non-responders selected for treatment by IHC alone (Fig. 3L).
- We then calculated HER2 expression only for transcripts with trastuzumab epitope (Fig. 3R).
- This epitope-resolution score is less correlated with IHC class than our 'all HER2 transcripts' score, with $\rho = 0.498$ for the score shown in Fig. 3R vs. $\rho = 0.630$ for the unfiltered score shown in Fig. 3L.
- Grouping epitope-specific scores by per-IHC class DESTINY-Breast04/06 response rates, a surprisingly consistent threshold emerges for response/non-response (Fig. 3R, dashed line), suggesting that trastuzumab-epitope expression may be a more direct predictor of ADC benefit than IHC scoring.

**We estimate 40% ultralow prevalence within IHC 0, and ultralow ORR of 61%, for ~24% ORR across IHC 0.*

Variable ADC target expression in whole blood enables toxicity monitoring

- ADC targets such as *HER2, HER3,* and *TROP2* are expressed by both healthy and tumor tissues, with high off-target expression potentially leading to toxicity and adverse events.
- To assess non-cancerous variation in target expression, we profiled 40 whole blood samples from control donors (see Methods) in six candidate ADC targets (some historic).
- We detected some level of healthy HER2 expression in 90% of control blood samples, with expression varying sample-to-sample by over an order of magnitude.
- Other ADC targets show a widely varying degree of non-cancerous expression in control blood (Fig. 4).
- For example, most control patients have high expression of CD44 in whole blood.
- An ADC targeting the CD44v6 isoform was shelved due to a phase I trial fatality[6].

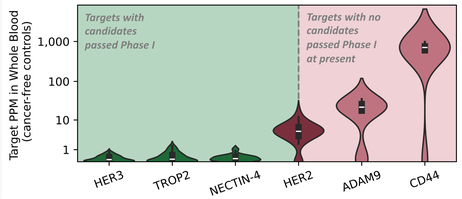

Figure 4: for six existing/prospective/historical ADC targets (x-axis), we show variation in PPM of expression of all transcripts of the given gene. Pseudo-log scale (linear in range 0-1) applied to y-axis.

Conclusions

- We have demonstrated the use of full-length transcriptome sequencing to distinguish trastuzumab-targeted HER2 expression from the broader HER2 expression detected by IHC.
- HER2 expression scores calculated across all transcripts correlate closely with IHC, confirming that our platform captures the same biology with finer resolution.
- This approach is applicable to any ADC target, even when IHC is unavailable/unreliable.
- By measuring trastuzumab epitope-specific expression, we identify a consistent response threshold across IHC classes, suggesting the potential to expand addressable populations among HER2 low/ultralow patients.
- Our platform also identifies alternative HER2 proteins that do not have the trastuzumab binding site, with implications for ADC response and resistance.
- We have shown, using whole blood samples from cancer-free patients, substantial variation in expression between ADC targets, and between patients for each target. This may indicate the need for further stratification on ADC targets or dose optimization.

References

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Disclosures

JB,TB,II,MB,JD,AR,MD,RH,HYC, and RK are employed and/or consult for Wobble Genomics.
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