

Isoform level RNA detection provides more detailed profiling of HER2 expression in breast cancer

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Background

- Accurate quantification of tumor HER2 expression is essential for guiding breast cancer therapy selection, as HER2 status directly determines eligibility for targeted therapies such as antibody-drug conjugates (ADCs).
- Current HER2 testing methods, primarily based on immunohistochemistry (IHC) and in-situ hybridization (ISH), often lack the precision to confidently distinguish HER2 statuses in borderline cases, which can lead to under- or over-treatment.
- Developing a more accurate and reliable HER2 quantification method will enable safer and more effective treatment choices, ensuring patients receive the most appropriate therapy while minimizing unnecessary exposure to side effects.
- Here we firstly confirm our platform's ability to determine HER2 status with high fidelity from breast cancer tissue and provide detailed isoform-level profiles.
- Next, we build upon previous work¹ to demonstrate the promise of our long-read RNA platform in determining HER2 status from blood, a substantial unmet need.

Methods

- We developed a novel platform that leverages long-read sequencing to capture full-length RNA and generate expression profiles at isoform resolution².
- We characterized transcriptome-wide expression of isoform features in blood samples, with a particular focus on HER2 and cancer-related genes.
- We used these expression signatures to stratify patients by HER2 status.
- Whole blood was used (instead of plasma or serum) to capture RNA from different cell types and larger extracellular vesicles. Blood was collected in PAXgene Blood RNA Tubes (Qiagen; 2.5ml per tube) and stored at -80°C (Figure 1).

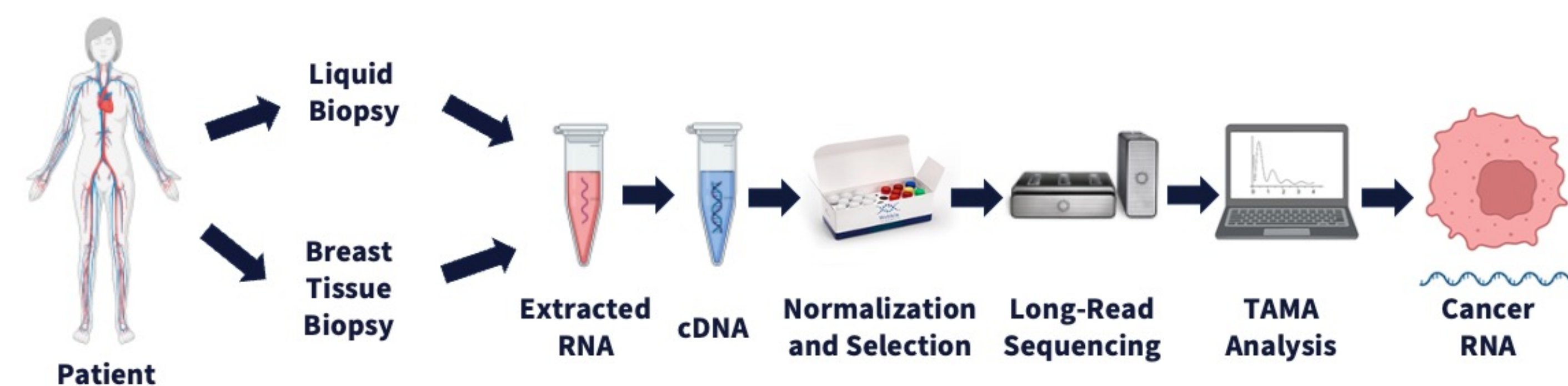


Figure 1: Wobble Genomics' sample processing workflow applying long-read transcriptomics to solid and liquid biopsy samples.

- Total RNA was extracted from the blood and converted into full-length cDNA using Wobble's long-range platform². Solid breast tumor samples were processed using Wobble's solid tissue platform.
- Resulting cDNA libraries were sequenced using Oxford Nanopore long-read sequencing, enabling annotation of complex isoform features.
- Sequencing data was analyzed using the TAMA software suite³ (Wobble Genomics).

Long reads in solid tumors correlate with HER2 IHC

- We analyzed 26 breast tumor samples with our solid tissue platform. These were assigned HER2 IHC scores of 0 ($n = 8$), 1+ ($n = 7$), 2+ ($n = 8$), or 3+ ($n = 3$) by pathologists.
- Normalized HER2 read counts showed substantial association with IHC subtypes (Spearman rank-correlation coefficient 0.714; $p = 4.17 \times 10^{-5}$; see Figure 2).
- HER2 positive (IHC 3+) samples had significantly higher HER2 scores than HER2 negative (IHC 0/1+/2+) samples ($p = 0.045$, by one-way ANOVA).

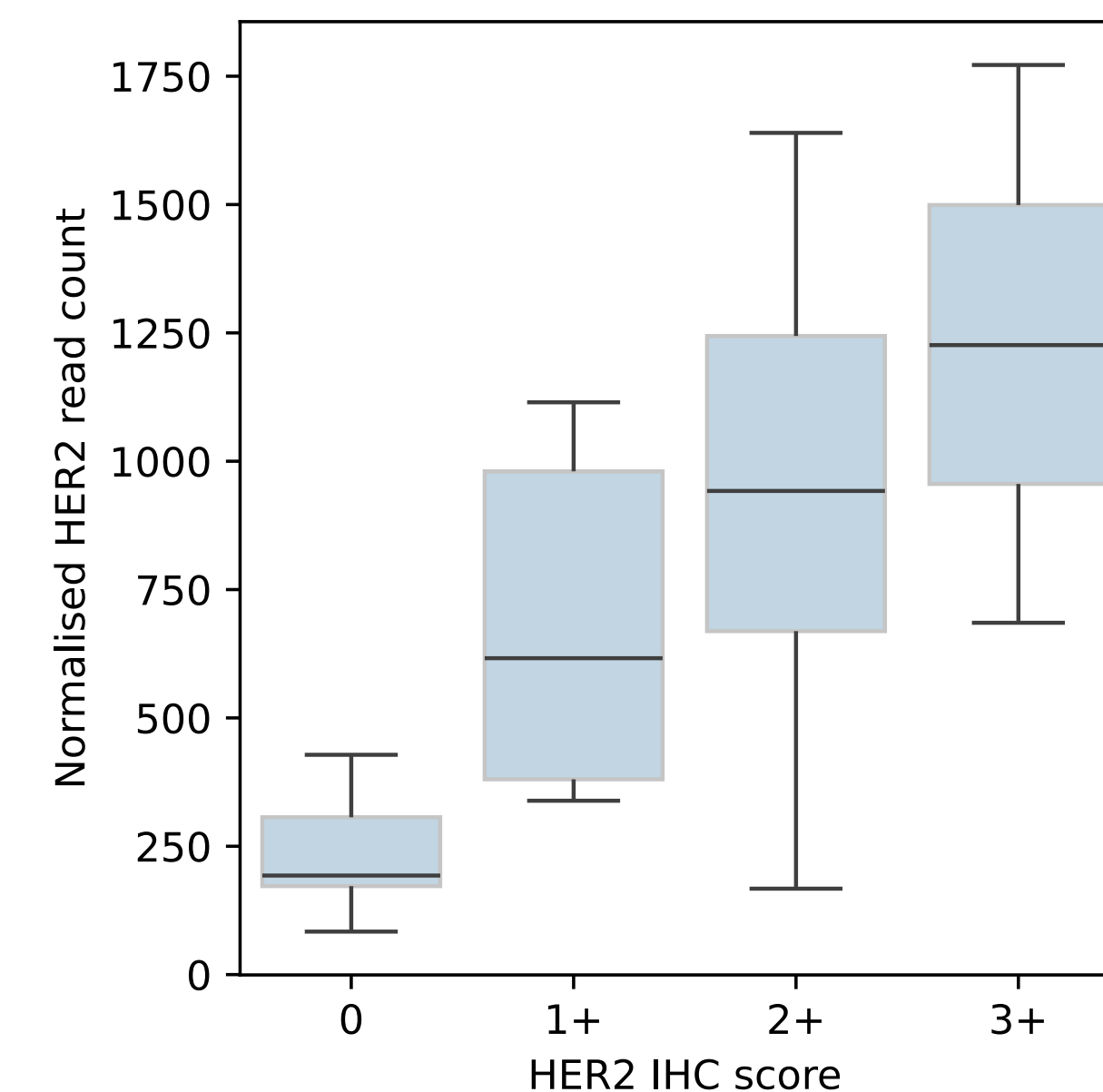


Figure 2: Per-IHC group normalized HER2 read counts from breast cancer tissue biopsy samples.

Long-read sequencing reveals diverse isoform features

- We identified 17,114 novel tumor-associated isoform features in 5,423 genes. These features were found in both our solid tumor samples and a cohort of blood samples from cancer patients, but never in a separate cohort of blood samples from non-cancer patients (novel = absent from *Ensembl* reference transcriptome⁴).
- These novel features were detected in up to 39% of blood samples from cancer patients and up to 100% of breast tumor biopsy samples (Figure 3).
- We curated a set of 1,223 cancer-related genes (based on the COSMIC Cancer Gene Census⁵, established ADC targets and genes with known relation to HER2 expression). We detected 1,381 of our novel features within 357 of these genes.

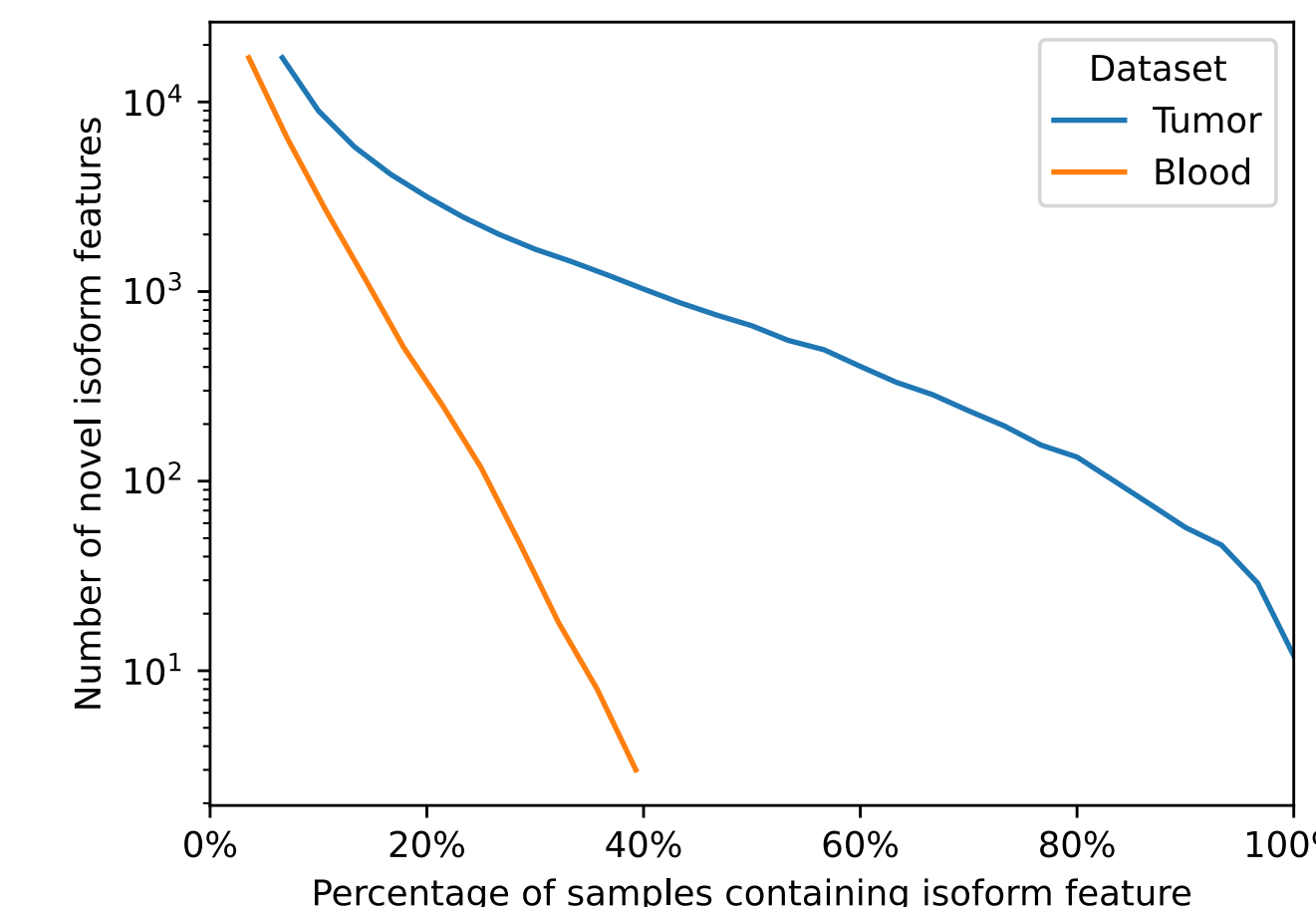


Figure 3: Distribution of prevalence of isoform features seen in cancer blood and tumor biopsy.

Long RNA detection from blood enables discrimination of HER2 expression

- We analyzed blood samples from early-stage breast cancer patients with HER2 negative (IHC 0/1+/2+) and HER2 positive (IHC 3+) status, alongside control blood samples from patients assessed for potential breast cancer and diagnosed cancer-free.
- We developed a scoring algorithm for HER2 subtype by analyzing isoform features from solid tumor samples, HER2-positive blood samples, control blood samples and matched pairs of blood samples from breast cancer patients pre- and post-surgery.
- We selected 103 blood samples meeting our QC requirements for use in cross-validation (20 IHC 0, 24 IHC 1+, 17 IHC 2+, 18 IHC 3+, and 24 matched controls).
- We applied our feature selection algorithm with 5-fold cross-validation, stratified by IHC group. We applied hyperparameter optimization for HER2 3+ vs HER2 0 separation in out-of-fold prediction.
- In Figure 4, we show out-of-fold HER2 score predictions for control, HER2 negative, and HER2 positive groups.
- AUROC for separation of HER2 positive and HER2 negative samples was 0.81 (Figure 5), with sensitivity of 78% for specificity of 85% (highlighted in red; $p = 6.90 \times 10^{-5}$; AUROC = area under receiver operating characteristic curve).

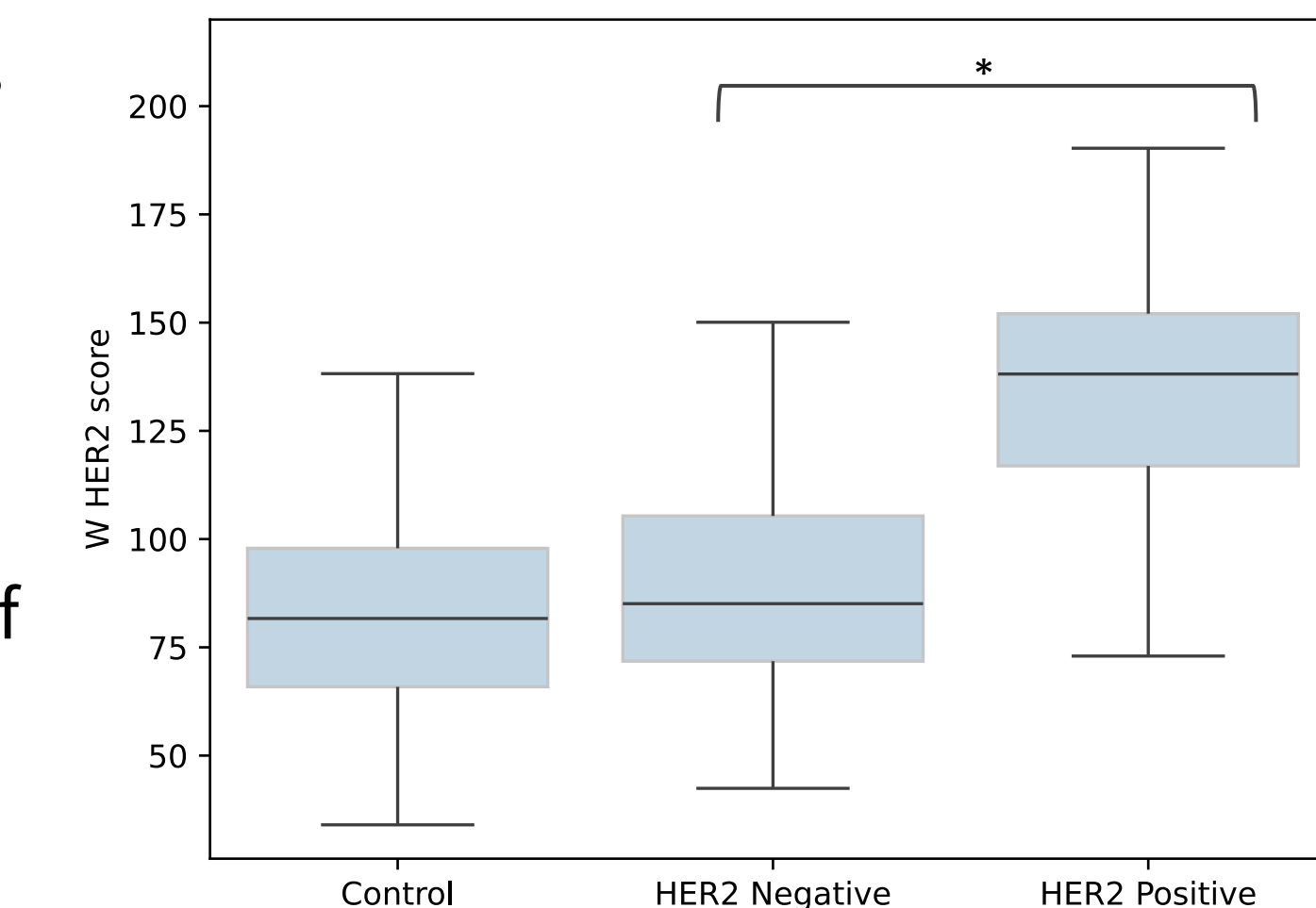


Figure 4: Wobble HER2 scores (out-of-fold predictions) per IHC category. Statistically significant difference denoted by “*”.

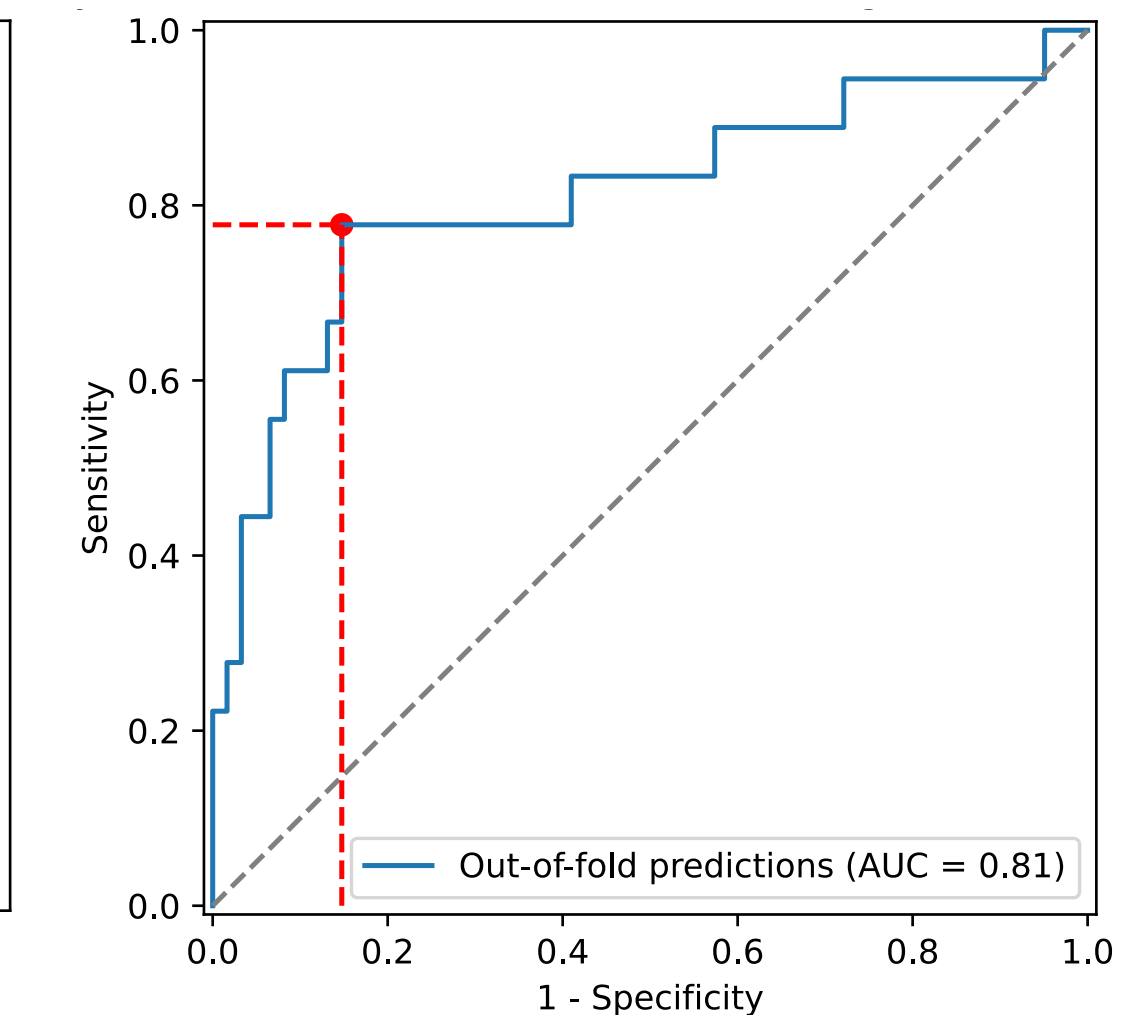


Figure 5: ROC curve for HER2 positive vs HER2 negative samples, from out-of-fold HER2 scores.

Conclusions

- Long-read RNA sequencing enables detailed profiling of the breast cancer transcriptome.
- Applying long-read RNA sequencing to solid tumor samples from breast cancer patients, HER2 expression correlates with HER2 IHC scores.
- We demonstrate the potential of long-read RNA sequencing for HER2 targeted therapy selection from blood.
- Our data provides new insights into transcript diversity and expression patterns, enabling improved diagnostic accuracy and refining treatment selection.

References

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Disclosures

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