Liquid biopsy determination of HER2 status in breast cancer: results from a novel epigenomic platform

[1]Heather A. Parsons*, [1]Sylvan C. Baca*, [2]Anthony D'Ippolito*, [2]Hyun-Hwan Jeong, [1]Kalie Smith, [1]Paolo Tarantino, [1]Melissa E. Hughes, [1]Kerry A. Sendrick, [2]Jonathan A. Beagan, [2]Aparna Gorthi, [2]Adrian B. Li, [2]Corrie A. Painter, [1]Ji-Heui Seo, [1]Elizabeth Grant, [2]Jamey Guess, [1]Matthew Davidsohn, [1]Gitanjali Lakshminarayanan, [1]Sarah Strauss, [1]Hunter Savignano, [1]John Canniff, [1]Brad Fortunato, [2]Matthew L. Eaton, [1]Toni K. Choueiri, [1]Sara M. Tolaney, [1]Matthew L. Freedman#, [1]Nancy U. Lin#

[1]Dana-Farber Cancer Institute, Boston, MA, [2] Precede Biosciences, Boston, MA

Background

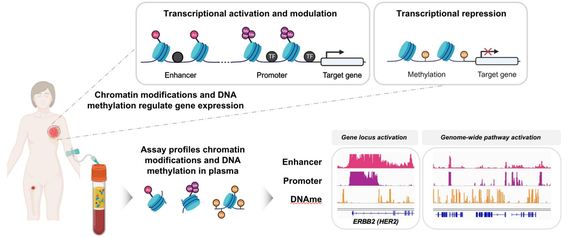
- Tumor HER2 and ER status are critical predictive biomarkers for patients with breast cancer.
- Challenges in obtaining biopsies, inter and intra-tumoral heterogeneity, and variability of IHC scoring (particularly for HER2-low) necessitate novel biomarkers for subtyping tumors from patients with metastatic breast cancer (MBC).
- Epigenomic profiling can identify transcriptional programs governing cancer phenotypes and their dynamic changes during the course of disease.[1]
- Here, we present a multimodal epigenomic liquid biopsy platform that offers a non-invasive alternative to tissue-based determination of transcriptionally regulated targets including HER2 and ER.

Methods

- We developed a multimodal epigenomic liquid biopsy approach by:
  - Developing a HER2 classifier via feature selection derived by epigenomically profiling circulating chromatin (enhancers and promoters) and DNA methylation (DNAme) from breast cancer cell lines
  - Cross-validating final classifier using a standard Leave-one-Out (LoO) scheme
- We profiled 1mL plasma from 130 patients with MBC enrolled in IRB-approved protocol 05-246 at Dana-Farber Cancer Institute (DFCI) or from 3 commercial biobanks, with blood samples drawn in Streck tubes within 6 weeks of tumor biopsy with HER2/ER IHC pathologist assessment
  - 109 samples yielded high-quality datasets across all analytes (114 promoter, 121 enhancer, and 126 DNAme datasets; see table below) and are included in the final analysis.

Source	HER2 IHC					ER IHC		ctDNA ≥ 3%
	0	1+	2+	2+/ISH+	3+	ER-	ER+	
DFCI	23	10	1	1	9	21	23	24
Vendor	31	12	0	6	16	26	33	40

Circulating chromatin and DNAme from 1mL of plasma

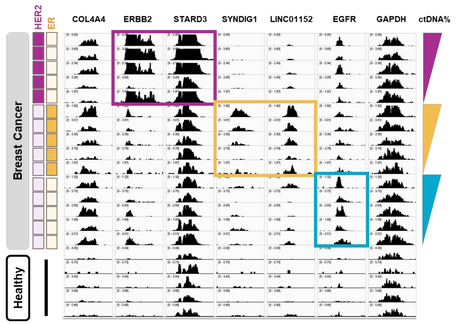


Epigenomic profiling from 1mL plasma reveals genome-wide transcriptional activation

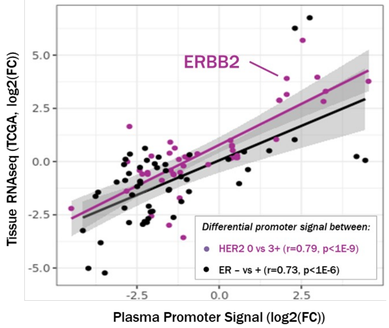
- This approach provides a real-time readout of gene regulation given 1) the short half-life of cfDNA and 2) the dynamic addition and removal of epigenomic modifications[1].
- Via immunoprecipitation of 1mL patient plasma, we isolate genome-wide epigenomic marks corresponding to activated promoters, activated enhancers, and methylated DNA from circulating chromatin
- Recovered DNA is quantified via high throughput sequencing to produce a readout for transcriptionally activated and repressed regions of the genome, allowing for interference of breast cancer receptor status

Genome-wide differential promoter signal sets correlate with mRNA expression in both HER2 and ER breast cancers

Promoter signatures of genes differentially regulated in HER2, ER, and triple negative breast cancers



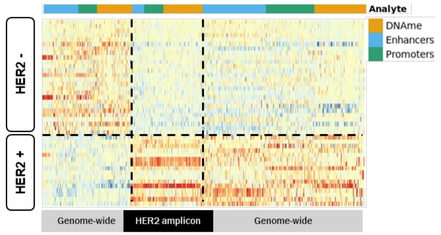
Genome-wide differential promoter signal sets correlate with mRNA expression in both HER2 and ER breast cancers



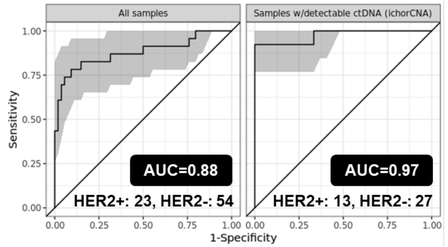
Promoter signatures of genes differentially regulated in breast cancer reveal distinct subtype-specific biology Left: Patient-specific (by row) promoter signals at genes representing differential transcriptional biology across breast cancer subtypes. Samples are grouped by HER2/ER IHC status and sorted by ichorCNA ctDNA fraction estimate within each group. The purple box designates loci specifically upregulated in HER2 3+ patients, the yellow box designates ER-positive upregulation, and the blue box highlights increased signal at the EGFR promoter in triple negative patients. Right: Correlation of TCGA mRNA expression and plasma-based promoter signals supports concordance between orthogonal approaches to profile transcriptional biology. The log2(fold change) (log2(FC)) of background-corrected promoter signal (+/- 1 kb from TSS) (x-axis) versus mRNA expression (TCGA RNAseq) (y-axis) resulting from the comparison of ER positive versus negative (black) and HER2 0 versus 3+ IHC-labeled (purple) patient samples. Only genes which exhibited significantly significant differential promoter signal are displayed. HER2 0 vs 3+ Pearson's r = 0.79, p-val = 1.22e-10; ER negative vs positive Pearson's r = 0.73, p-val = 9.41e-7.

HER2 plasma-derived classifier correlates strongly with tissue phenotype in HER2 3+ vs 0

HER2 +/- differential loci detected at amplicon and genome-wide

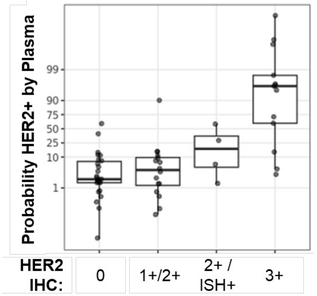


Robust cross-validated HER2 classifier



Robust performance of HER2 3+ versus HER2 0 multimodal classifier Left: Genomic loci with differential signal (FDR ≤ 0.05, log2 fold-change ≥ 1) between HER2 3+ and HER2 0 patients at enhancers, promoters, and sites of DNAme. Signal has been normalized for ctDNA % and the z-score was calculated for each locus. Right: Genome-wide enhancer, promoter, and DNAme signal was used to train a regularized logistic regression model to classify HER2 IHC status (0 vs 3+). Performance was assessed via AUC in a leave-one-out cross-validation scheme. Performance was stratified by whether ctDNA was detectable using ichorCNA (>3%) on patient-matched low-pass whole genome sequencing data[2].

Application of HER2 3+/0 classifier to other HER2 states

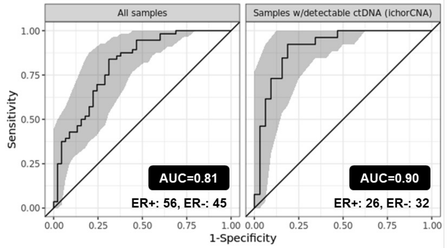


HER2 stratification leveraging multimodal analyte HER2 3+ vs 0 classifier

- Promising efficacy of HER2 ADCs in HER2-low population highlight significant need to identify patients
- OR in DAISY: 3+ = 71%; 2+ = 35%; 1+ = 39%; 0= 30%
- Additional samples with same antibody, central review & quantitative measure of HER2 will be included for HER2-low classifier development
- Further work will build on these results to develop a robust HER2-low classifier

HER2-low sample predictions from HER2 3+ versus 0 model indicates potential for HER2-low classifier development Using the HER2 3+ vs 0 classifier described previously, the probability of HER2 positivity was determined for samples classified as HER2-low by IHC, demonstrating a positive association of the probability of HER2 positivity and IHC score.

Plasma-based ER classifier strongly correlates with tissue



Cross-validated multimodal ER classifier generated from 116 loci (48% enhancers)

Development of an ER classifier from same samples used to generated HER2 classifier demonstrates robust performance Genome-wide enhancer, promoter and DNAme signal was used to train a regularized logistic regression model to classify ER IHC status. Performance was assessed via AUC in a leave-one-out cross-validation scheme. Performance was stratified by whether ctDNA was detectable using ichorCNA on patient-matched low-pass whole genome sequencing data.

Conclusions

- In this retrospective study profiling 1mL plasma from 130 patients with MBC, we provide promising proof-of-concept from a novel multimodal liquid biopsy evaluating genome-wide tumor transcriptional regulation
- We demonstrate the ability to reveal biology unique to HER2+, HER2-, ER+ and ER- metastatic breast cancer in a non-invasive manner
- Biomarker gold standard challenges include tissue/plasma biopsy concordance impacted by intra- and inter-tumor heterogeneity and inter-pathologist variability in IHC assessment & variability in HER2 antibody
- Robust classifiers distinguishing between HER2 3+ vs HER2 0 and ER+ vs ER- were developed, with potential for classification of HER2-low demonstrated and ongoing.
- This method is compatible with standard collection protocols of plasma in Streck or EDTA tubes and shows promise as a non-invasive means to assess and HER2 and ER status in patients with MBC in clinical practice

References

1. Baca SC, Seo JH, Davidsohn MP, Fortunato B, Semaan K, Sotudian S, Lakshminarayanan G, Diossy M, Qiu X, El Zarif T, Savignano H, Cannifff J, Madueke I, Saliby RM, Zhang Z, Li R, Jiang Y, Taing L, Awad M, Chau CH, DeCaprio JA, Figg WD, Greten TF, Hata AN, Hodi FS, Hughes ME, Ligon KL, Lin N, Ng K, Oser MG, Meador C, Parsons HA, Pomerantz MM, Rajan A, Ritz J, Thakuria M, Tolaney SM, Wen PY, Long H, Berchuck JE, Szallasi Z, Choueiri TK, Freedman ML. Liquid biopsy epigenomic profiling for cancer subtyping. Nat Med. 2023 Nov;29(11):2737-2741. doi: 10.1038/s41591-023-02605-z. Epub 2023 Oct 21. PMID: 37865722.Our work demonstrates the ability to reveal biology unique to HER2+, HER2-, ER+ and ER- metastatic breast cancer in a non-invasive manner

2. Adalsteinsson, V.A., Ha, G., Freeman, S.S. *et al.* Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. *Nat Commun* **8**, 1324 (2017). https://doi.org/10.1038/s41467-017-00965-y

Contact

Heather A. Parsons, MD MPH
Dana-Farber Cancer Institute
Email: heather_parsons@dfci.harvard.edu

Acknowledgments

Figure created with BioRender.com

San Antonio Breast Cancer Symposium
December 5-9, 2023

This presentation is the intellectual property of the author/presenter. Contact them at heather_parsons@dfci.Harvard.edu for permission to reprint and/or distribute.

© Poster Template by Genigraphics® 1.800.790.4001 www.genigraphics.com