Classification of HER2 Status Across Multiple Cancers Using Epigenomic Profiles from A Novel Liquid Biopsy Assay

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BACKGROUND

Trastuzumab deruxtecan recently received FDA accelerated approval for any HER2-positive solid tumors.

Reliable assessment of HER2 expression across solid tumors is critically needed.

Current IHC/ISH HER2 scoring faces challenges like scoring discordance, tumor heterogeneity, and lack of sufficient tissue for evaluation.

A novel liquid biopsy (LBx) platform using epigenomic signatures from 1mL of plasma offers a minimally invasive approach for HER2 status classification and patient selection across cancers.

METHODS

We selected 179 samples from 172 patients with advanced breast (BC), gastro-esophageal (GEA) and ovarian (OV) cancers who had associated HER2 status scored from tissue-based IHC/ISH according to ASCO/CAP guidelines (first table).

Samples were taken at baseline or at progression and those with detectable cell free DNA (BC assessed by proprietary method, LoD = 1%; non-BC assessed by ichorCNA, LoD = 3%, Adalsteinsson et al.) were profiled for genome-wide epigenomic signals across histone modifications associated with active enhancers, promoters and DNA methylation.

We quantified gene-regulatory signal at the HER2 locus using literature-supported regulatory element annotations, and compared this signal to mRNA tissue expression in patients with matched RNA-seq.

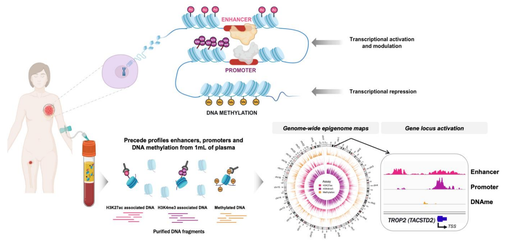
A genome-wide regularized regression model developed to classify HER2 status in BC cell lines and was refined and validated for HER2 status prediction for each patient cohort (HER2+=3+, 2+/ISH+; HER2-=2+/ISH-, 1+, 0).

The BC cell-line derived HER2 classifier was refined to incorporate GEA specific features and was also tested directly on OV patients.

Performance of classifiers was assessed via AUC in a leave-one-out cross-validation schema.

RESULTS

Figure 1. Comprehensive Epigenomic Platform Offers Dynamic Resolution into Target and Pathway Biology from 1mL of Plasma

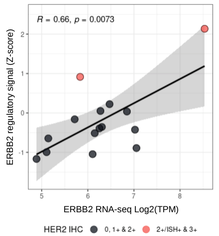


Cell free DNA derived from tumors exists in circulation as chromatin fragments that faithfully maintain tumor-associated epigenetic modifications on the histones and DNA. Antibodies against H3K27ac marking active enhancers, H3K4me3 marking active promoters and DNA methylation are used to enrich for associated DNA fragments from 1mL plasma and sequenced to define genome-wide epigenomic maps that capture the underlying transcriptional state of the tumor cells (Baca et al.).

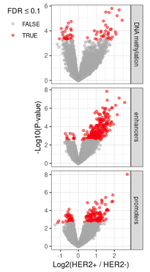
PATIENT COHORTS	Breast Cancer		Ovarian Cancer		Gastroesophageal Cancer	
	Samples	Patients	Samples	Patients	Samples	Patients
Samples processed	119	111	14	13	46	46
Samples w/detectable ctDNA	105	98	4	4	14	14

Figure 2. Breast Cancer *ERBB2* RNA-seq Expression in Matched Biopsies Correlates with Plasma Gene-regulatory Signal At *ERBB2* Locus

Precede plasma HER2 quantification correlates with tissue RNA-seq



Regions outside the HER2 amplicon with differential signal between IHC HER2+ & HER2-

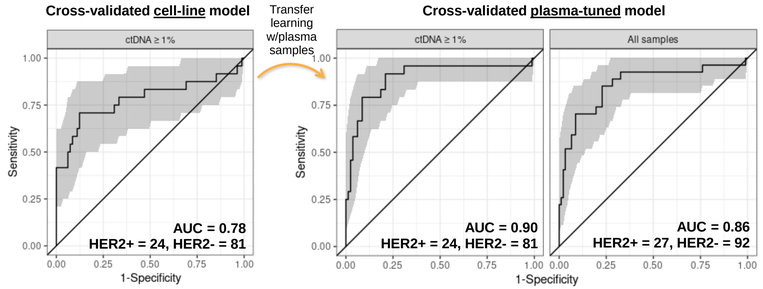


Differentially active promoters & enhancers outside HER2 amplicon are biologically relevant to HER2 signaling

Gene	HER2 IHC	Involvement in HER2 Signaling (PMID)
CBX2	HER2+	30972192
KRT19	HER2+	25342465
DUSP4	HER2+	29100381
CCND1	HER2+	10611246
PIK3CA	HER2+	29760043
PIK3C2B	HER2+	29760043
TFAP2A	HER2+	37291585
AKT2	HER2-	31752925

Left, Using a subset of our cohort that had matched tissue RNA-seq we correlated ERBB2 mRNA expression in tissue to the gene-regulatory signal at the ERBB2 locus measured in the plasma. These gene-regulatory quantifications were used to classify HER2 IHC 3+/2+ISH+ from 2+/1+/0 with a Leave One Out (LOO) AUC of 0.78 (ctDNA ≥ 3%). Middle, In our breast cancer plasma samples with detectable ctDNA we observe numerous loci across promoters, enhancers and DNA methylation that have statistically significant signal between HER2 IHC 3+/2+ISH+ and 2+/1+/0 patients, indicating the potential of using regions outside the HER2 amplicon for IHC classification. *Right,* For all the promoters and enhancers discovered to be differential using plasma from HER2+ and HER– patients, we linked them to their closest gene, and we found that many of these genes have literature support for their involvement in HER2 signaling.

Figure 3. An Epigenomic Genome-wide HER2+ Vs HER2- Breast Cancer Classifier was Developed Using Information from Both Cell Lines and Plasma

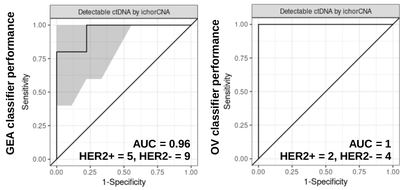


	HER2 Amplicon			Outside of *HER2* Amplicon		
Model	Enhancers	Promoters	DNAme	Enhancers	Promoters	DNAme
Cell-line	6	4	0	23	8	15
Plasma-tuned	7	4	1	36	13	23

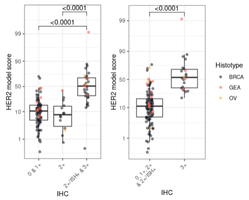
Epigenomic data from 26 breast cancer cell lines (8 HER2 IHC 3+ and 18 HER2 IHC 0) were used to train a lasso logistic regression model. Loci with robust regulatory signal in our breast cancer patient cohort were selected in a LOO schema and predictions of held-out samples yielded a classification AUC of 0.78 (HER2 IHC 3+/2+ISH+ vs 2+/1+/0). Cell line models were then tuned using regulatory signal information from our breast cancer plasma samples, yielding an AUC of 0.9 in samples with ctDNA ≥ 1%. We also observed an AUC of 0.86 across all samples, implying that some samples that fall below our ctDNA LoD but still have ctDNA are being classified correctly.

Figure 4. Epigenomic Liquid Biopsy Can Classify HER2 Status in Other Tumor Types

The HER2 breast cancer classifier can be used to initiate training of HER2 classifiers in other tumor types



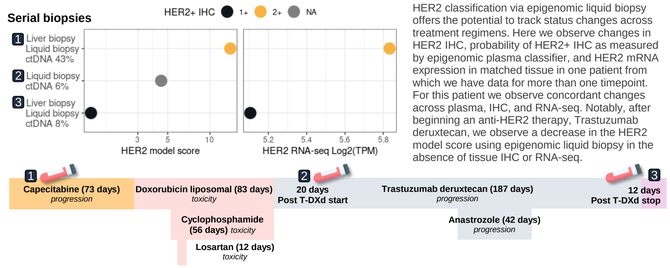
Pan-cancer HER2 classification by epigenomic liquid biopsy correlates with HER2 IHC



Left, To determine the utility of our assay to distinguish HER2 IHC status (3+/2+ISH+ vs 2+/1+/0) we analyzed plasma samples from patients with gastroesophageal (GEA) or ovarian (OV) cancer. We used GEA samples to tune our original breast cancer cell line model in a LOO schema and observed an AUC of 0.96 in samples with detectable ctDNA (ichorCNA). For OV we observe a classification AUC of 1. *Right*, When aggregating our HER2 IHC predictions (3+/2+ISH+ vs 2+/1+/0) across indications, we observe a linear trend of our model probabilities with HER2 IHC status. Also, though trained on 3+/2+ISH+, we observe a statistically significant (Wilcoxon test) separation in model probabilities between IHC 3+ samples and those with other IHC scores, demonstrating the potential for patient stratification for therapies that require HER2 IHC 3+ for inclusion.

RESULTS

Figure 5. HER2 Classification Via Epigenomic LBx Tracks Longitudinal Response To Trastuzumab deruxtecan, Coincident with RNA-seq and IHC

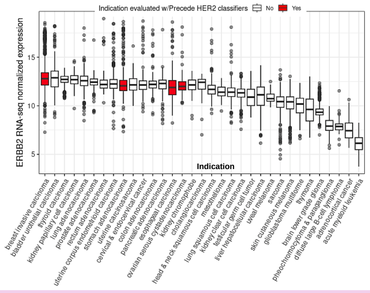


HER2 classification via epigenomic liquid biopsy offers the potential to track status changes across treatment regimens. Here we observe changes in HER2 IHC, probability of HER2+ IHC as measured by epigenomic plasma classifier, and HER2 mRNA expression in matched tissue in one patient from which we have data for more than one timepoint. For this patient we observe concordant changes across plasma, IHC, and RNA-seq. Notably, after beginning an anti-HER2 therapy, Trastuzumab deruxtecan, we observe a decrease in the HER2 model score using epigenomic liquid biopsy in the absence of tissue IHC or RNA-seq.

Figure 6. Plasma-based Epigenomic Classification of HER2 Could Be Applied to Other Indications with Evidence of HER2 Expression

HER2 RNA-seq expression (TCGA Pan-Cancer indications)

The recent label expansion of trastuzumab deruxtecan to include all HER2-positive (IHC 3+) solid tumors expands treatment options across various solid tumor types. As the landscape of tumor-agnostic therapies broadens, particularly those requiring immunohistochemistry (IHC) for biomarker identification, the demand for tissue samples will intensify. A comprehensive multi-analyte blood assay capable of providing genome-wide expression could enable a minimally invasive multiplexed strategy to identify biomarkers that currently depend on tissue-based analyses. We examined various tumor types using RNA-seq expression data from the TCGA Pan-Cancer Atlas, aiming to highlight potential candidates that could benefit from this therapy. However, such application necessitates testing for HER2 expression, which is not currently included in the standard of care.



CONCLUSIONS

This retrospective study profiling 1mL plasma from 172 patients with advanced breast, gastroesophageal, and ovarian cancers demonstrates that HER2 classification using comprehensive epigenomic signals can be applied across multiple cancers.

This method may overcome variability due to tumor heterogeneity and inter-pathologist discordance.

A standardized approach across all solid tumors will enable harmonized patient selection for Trastuzumab deruxtecan.

With further development, our genome-wide profiling approach could alleviate clinical constraints associated with multiple tissue-based HER2 scoring assays (IHC/ISH) and enable longitudinal monitoring of HER2 status during therapy.

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