A Novel Epigenomic Profiling Assay Reveals Disease Biology and Therapeutic Targets in Gastro-esophageal Cancer from 1mL of Plasma

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ABSTRACT # 8833

INTRODUCTION

- Gastro-esophageal adenocarcinoma (GEA) is an aggressive and heterogeneous disease with a poor prognosis.
- Targeted therapies have failed largely due to challenges associated with biopsy collection, tumor antigen heterogeneity, and accurately distinguishing transcriptionally distinct esophageal, GE junction or gastric tumor types.
- We developed a novel, multimodal liquid biopsy assay that profiles genome-wide transcriptional activation[1].
- Here, we deploy this assay to define clinically relevant insights in a cohort of GEA patients.

METHODS

We profiled 107 1mL plasma samples from 65 patients with gastroesophageal cancer enrolled in an IRB-approved protocol Massachusetts General Hospital (MGH). 56 samples represented pre-treatment blood draws with matched HER2 IHC pathologist assessment. 46 pre-treatment samples yielded high-quality datasets across all analytes (promoter, enhancer, DNAme). 15 samples had detectable ctDNA by ichorCNA estimation[2]. HER2 IHC, staging, and histology grade breakdown of this cohort is below.

HER2 IHC (all samples, n=46/ detectable ctDNA by ichor, n=15)			
0	1+	2+	3+
26 / 7	5 / 2	8 / 2	7 / 4

Staging at Sample Collection (all samples, n=46/ detectable ctDNA by ichor, n= 15)	
III	IV
27 / 3	19 / 12

Histology grade (all samples, n=46/ detectable ctDNA by ichor, n=15)		
Poorly Differentiated or Grade 3	Moderate-poorly Differentiated or Grade 2	Not Reported
12 / 4	11 / 2	23 / 9

We developed a HER2 classifier trained on epigenomic features (enhancers, promoters, DNAme peaks) which enabled classification of HER2 ± cancer cell lines, as previously presented (Parsons et al., SABCS 2023)[3].

- We further tuned this model for application to gastroesophageal cancer plasma samples.
- Classifier was cross-validated using standard leave-one-out scheme.

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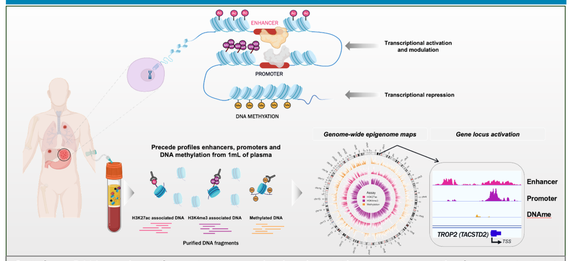
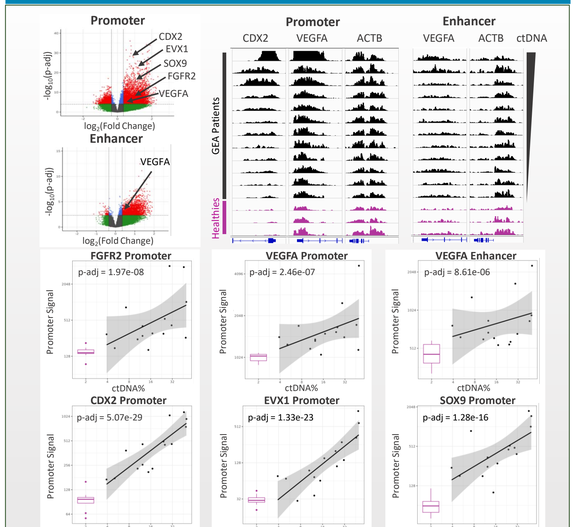
RESULTS

Comprehensive epigenomic platform offers dynamic resolution into target and pathway biology from 1 mL of plasma



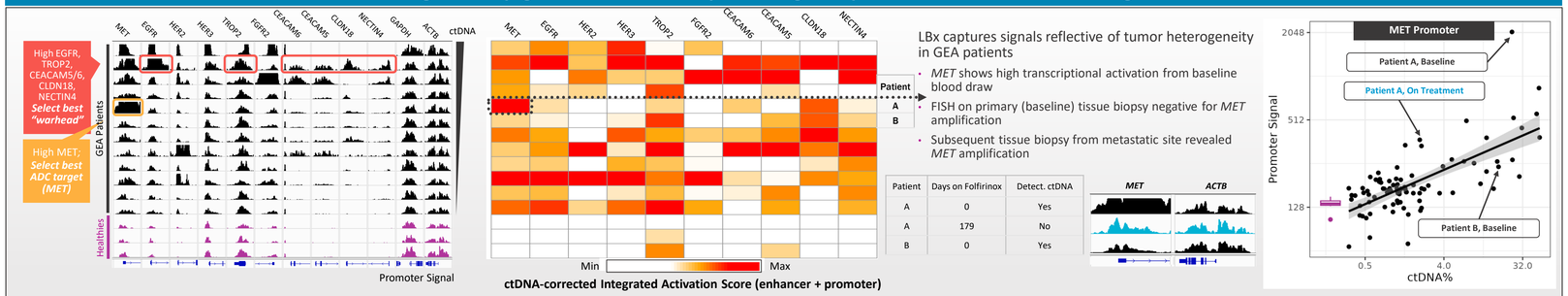
Cell free DNA derived from tumors exist in circulation as chromatin fragments that faithfully maintain tumor-associated epigenetic modifications on the histones and DNA. Antibodies against H3K27ac marking active enhancers, H3K4me3 marking active promoters and DNA methylation are used to enrich for associated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

Figure 1: Plasma-based epigenomic activation signal at GEA marker genes



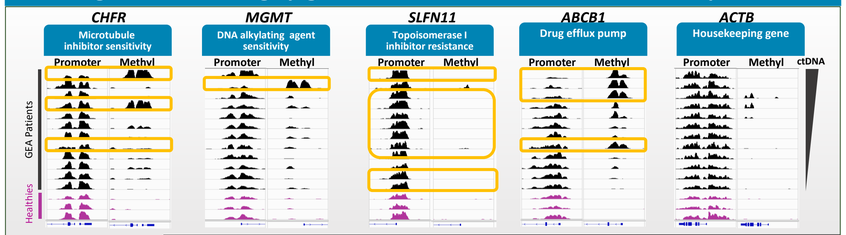
Genome-wide promoter and enhancer regions were assessed for correlation of signal with ichor ctDNA% estimates. ***Top left:*** Volcano plot showing genes with positive fold change display increased signal in GEA cancer samples (red). ***Top Right, Bottom:*** Promoter tracks and scatterplots of promoter/enhancer signal vs estimated ctDNA% (above) are displayed for select growth factors (*FGFR2, VEGFA*) and transcription factors (*CDX2, EVX1, SOX9*) previously associated with gastroesophageal cancer.

Figure 2: Epigenomic activation panel to guide patient selection for ADC targets

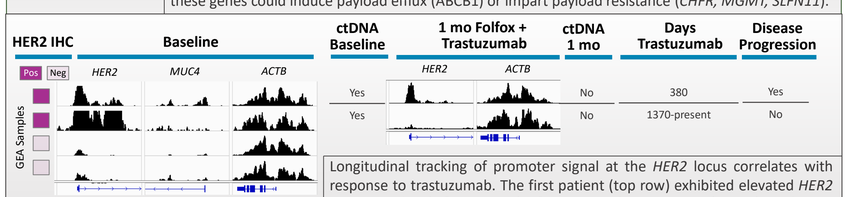


Left: Patient-specific (by row) promoter signals at genes representing select ADC targets (plus housekeeping genes *GAPDH/ACTB*). Samples are sorted by ichor ctDNA fraction estimate. ***Middle:*** Enhancer and promoter signals for each gene were ctDNA-corrected and combined into an epigenomic activation score, represented on a white (low) to red (high) gradient. ***Right:*** LBx detection of elevated *MET* signal initially absent from tissue biopsy of Patient A. Elevated *MET* promoter signal identified by promoter tracks and promoter signal vs ctDNA linear residual (scatter plot). Patient B is a high ctDNA GEA patient sample with moderate-low MET promoter signal included for comparison.

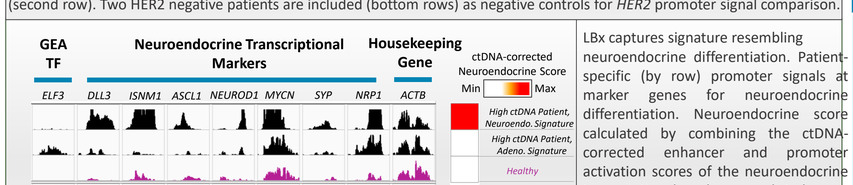
Figure 3: Evaluating epigenomic status of modulators of ADC response



Patient-specific (by row) promoter and DNA methylation signals at genes known to modulate effectiveness of ADC / chemotherapy payloads (plus housekeeping gene *ACTB*). Altered expression of these genes could induce payload efflux (ABCB1) or impart payload resistance (*CHFR, MGMT, SLFN11*).

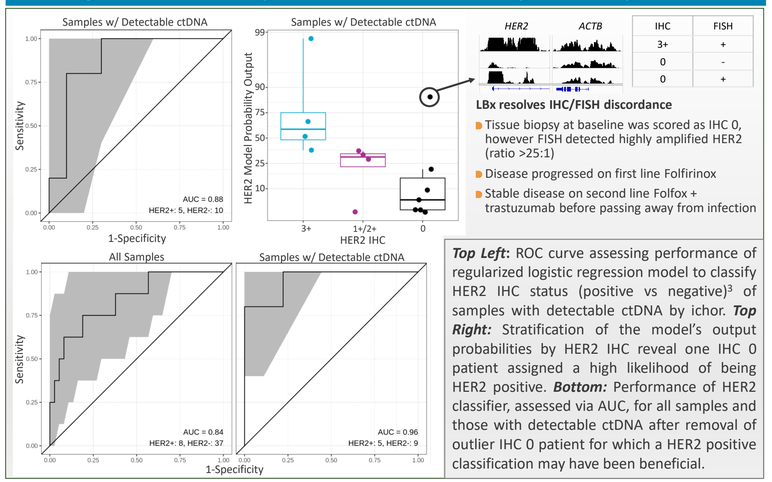


Longitudinal tracking of promoter signal at the *HER2* locus correlates with response to trastuzumab. The first patient (top row) exhibited elevated *HER2* promoter signal after 1 month of trastuzumab treatment, elevated *MUC4* (trastuzumab resistance marker) promoter signal at baseline, and quicker disease progression compared with the second patient (second row). Two HER2 negative patients are included (bottom rows) as negative controls for *HER2* promoter signal comparison.



LBx captures signature resembling neuroendocrine differentiation. Patient-specific (by row) promoter signals at marker genes for neuroendocrine differentiation. Neuroendocrine score calculated by combining the ctDNA-corrected enhancer and promoter activation scores of the neuroendocrine transcriptional marker genes listed.

Figure 4: Multi-analyte HER2 classifier robustly stratifies patients



Top Left: ROC curve assessing performance of regularized logistic regression model to classify HER2 IHC status (positive vs negative)[3] of samples with detectable ctDNA by ichor. ***Top Right:*** Stratification of the model's output probabilities by HER2 IHC reveal one IHC 0 patient assigned a high likelihood of being HER2 positive. ***Bottom:*** Performance of HER2 classifier, assessed via AUC, for all samples and those with detectable ctDNA after removal of outlier IHC 0 patient for which a HER2 positive classification may have been beneficial.

CONCLUSIONS

- Here, we present a novel liquid biopsy approach that leverages comprehensive epigenomic profiling from 1 mL of plasma, an approach that represents an advance over current tissue and liquid based methods.
- Our genome-wide approach enabled direct comparison of the activation status of GEA-relevant ADC target genes such as *HER2*, *HER3*, and *MET* along with genes indicating sensitivity to warhead class, highlighting potential for use in patient selection.
- Using a HER2 classifier trained on enhancer, promoter, and DNA methylation profiles, we accurately stratify patients based on IHC/FISH status, identifying those who may benefit from *HER2*-directed therapy.
- Our minimally invasive approach enables longitudinal expression profiling of therapy related dynamics, thereby informing rational sequential therapeutic strategies.

References:
1.Baca SC, Seo JH, Davidsohn MP *et al*. Liquid biopsy epigenomic profiling for cancer subtyping. *Nat Med* 29, 2737–2741 (2023).
2.Adalsteinsson VA, Ha G, Freeman SS *et al*. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. *Nat Commun* 8, 1324 (2017).
3.Parsons H, Baca S, D'Ippolito A, *et al*. Liquid biopsy determination of HER2 status in breast cancer: results from a novel epigenomic platform. Presented at the 2023 San Antonio Breast Cancer Symposium (2023).

Presenting Author Disclosures: Dr. Klempner reports consulting/advisory fees from Merck, BMS, Eli Lilly, Natera Oncology, Pieris, Daiichi-Sankyo, Sanofi-Aventis, Foundation Medicine, and stock/equity in Turning Point Therapeutics.

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