ASCO 2025

Abstract #1081

Cell-free circulating chromatin profiles enable epigenomic characterization of mechanisms of response and resistance to sacituzumab govitecan in breast cancer

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BACKGROUND

- Sacituzumab govitecan (SG), a TROP2-directed antibody-drug conjugate (ADC) with a topoisomerase-I inhibitor payload, demonstrated significantly improved progression-free survival (PFS) and overall survival (OS) vs chemotherapy in patients with hormone receptor-positive/HER2-negative (HR+/HER2-) metastatic breast cancer (mBC)[1].
- Predictive biomarkers of response and resistance to treatment remain an unmet clinical need.
- SACI-IO HR+ (NCT04448886) is a randomized, open-label, phase II study designed to evaluate the efficacy and safety of SG with or without pembrolizumab in patients with HR+/HER2- mBC.
- We applied a novel comprehensive epigenomic liquid biopsy assay to characterize tumor-specific transcriptional activation of relevant genes of interest and resistance mechanisms from plasma in patients enrolled in the SACI-IO HR+ trial.

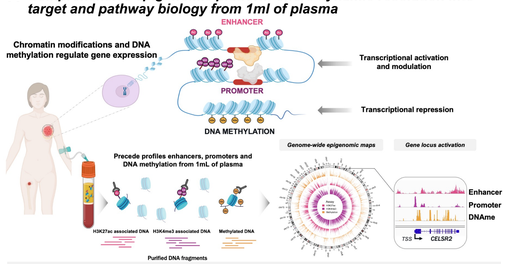
METHODS

- Baseline plasma samples were collected from patients with HR+/HER2- mBC who enrolled in SACI-IO HR+.
- Genome-wide signals from promoters, enhancers and DNA methylation were profiled from 1mL of plasma from 95 patients, of which 80 patients met the assay quality control thresholds and ctDNA metrics required for downstream analysis (ctDNA ≥0.5%, N_{SG}=42, $N_{SG+pembro}$=38).
- Epigenomic and RNA-seq datasets from breast cancer models were used to train a model to predict *TROP2* expression (r=0.66, P<0.01) and tested for association with PFS.
- GSVA was used to score samples for HALLMARK gene set activities using gene-proximal epigenomic signals and tested for association of those activities with PFS via CoxPH models, with baseline ctDNA fraction as an additional covariate.
- Statistical significance was determined based on improved model fit compared to ctDNA alone to account for any contribution due to circulating tumor fraction.

RESULTS

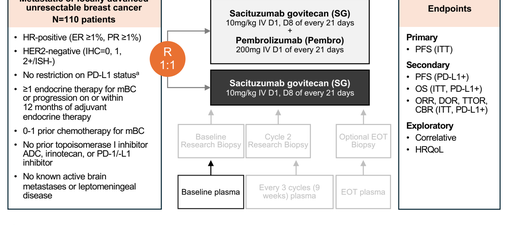
Figure 1: Comprehensive epigenomic profiling from 1 mL of plasma

A ***Comprehensive epigenomic platform offers dynamic resolution into target and pathway biology from 1ml of plasma***



Cell free DNA derived from tumors exist in circulation as chromatin fragments that maintain tumor-associated epigenetic modifications on the histones and DNA. Antibodies against active enhancers, active promoters and DNA methylation are used to enrich for associated DNA fragments from 1mL plasma and sequenced to capture the underlying transcriptional state of the tumor cells[2].

B ***SACI-IO HR+ study schema***



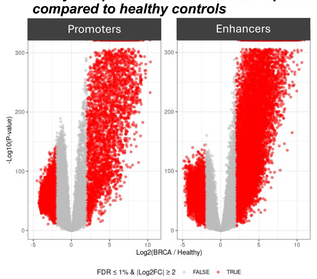
C ***Baseline characteristics***

COHORT DESCRIPTION	SG (N=52)	SG+Pembro (N=52)	ITT (N=104)
Baseline plasma samples evaluated, n	**42**	**38**	**-**
ctDNA%, median (range)	15 (0.7-61)	17 (1-80)	-
Patient characteristics			
Age in years, median (range)	58.5 (31-80)	55.5 (31-81)	57.0 (27-81)
Race, number (%)			
White	35 (83.3)	30 (78.9)	84 (80.8)
Black or AA	3 (7.1)	3 (7.9)	7 (6.7)
Asian	0 (0.0)	3 (7.9)	5 (4.8)
American Indian or Alaskan Native	0 (0.0)	0 (0.0)	1 (1.0)
Other	4 (9.5)	2 (5.3)	7 (6.7)
Disease characteristics			
ER status			
≥10%	40 (95.2)	37 (97.4)	99 (95.2)
1-9%	1 (2.4)	1 (2.6)	3 (2.9)
Unknown	1 (2.4)	0 (0.0)	2 (1.9)
Presentation at mBC diagnosis			
De novo mBC	10 (23.8)	8 (21.1)	23 (22.1)
Recurrent mBC	32 (76.2)	30 (78.9)	81 (77.9)
Metastasis at baseline, number (%)			
Liver	32 (76.2)	31 (81.6)	81 (77.9)
Brain	5 (11.9)	4 (10.5)	9 (8.7)
Prior treatment regimens, n (%)			
Neo-/adjuvant chemotherapy[a]	24 (75.0)	21 (70.0)	56 (69.1)
CDK4/6 inhibitor in any setting	36 (85.7)	34 (89.5)	92 (88.5)
Chemotherapy regimen (one line)	21 (50.0)	19 (50.0)	51 (49.0)

[a] Patients diagnosed with *de novo* stage IV breast cancer excluded from denominator.

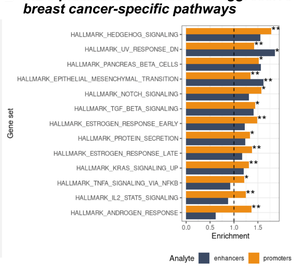
Figure 2: The mBC plasma epigenome is enriched for breast cancer-associated biology

A ***Study samples have 1000s of mBC-specific regulatory loci compared to healthy controls***

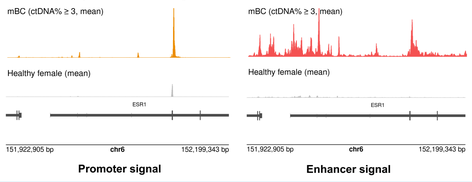


A Consensus loci for promoter and enhancers were tested for differential signal in mBC cohort compared to healthy female volunteer plasma used as controls (N=53). Signal at each loci was normalized for ctDNA% in mBC samples and compared to a cohort of healthy female samples using negative binomial generalized linear models.

B ***mBC plasma is enriched with aggressive breast cancer-specific pathways***



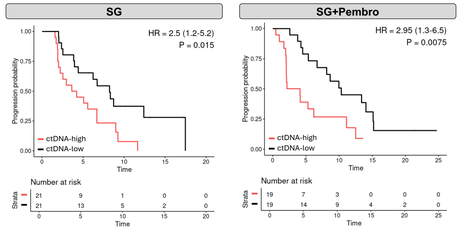
C ***Samples have increased promoter and enhancer signal at ESR1 compared to healthy controls***



B Differential loci identified in (A) with a Log2(fold-change) >5 and FDR ≤1% were used to test for enrichment near genes in HALLMARK gene sets using GREAT[3]. All sites used in differential testing were used as the background set for enrichment calculations. *FDR ≤10%, **FDR ≤5%.

C Promoter and enhancer signal at the *ESR1* locus was normalized for ctDNA, averaged across the mBC cohort (≥3% ctDNA) and compared to the average signal across a cohort of female healthy controls (N=53).

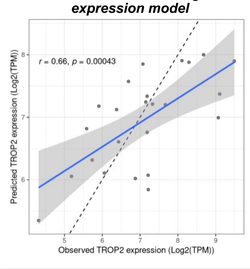
Figure 3: Higher baseline ctDNA fraction is associated with worse PFS



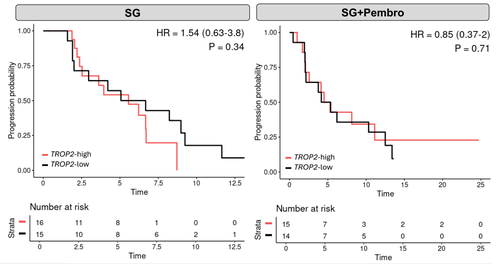
- Patient plasma samples from SG and SG+Pembro cohorts were stratified into ctDNA-high and ctDNA-low groups using the median ctDNA fraction as a cutoff.
- Association of ctDNA groups with PFS was tested via Cox proportional hazards (CoxPH) model (hazard ratios [HR] and associated P-values displayed).
- HR values measure relative risk of ctDNA-high group compared to ctDNA-low group. Baseline tumor fraction was prognostic in both treatment arms (P<0.01).

Figure 4: Higher baseline predicted *TROP2* expression is not significantly associated with PFS

A ***Performance of TROP2 gene expression model***



B ***TROP2 expression is not associated with PFS***

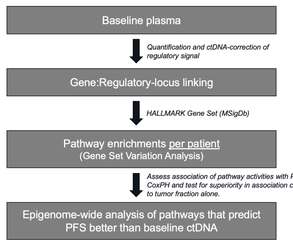


A Genome-wide plasma epigenomic features associated with *TROP2* expression in breast cancer models were used to train a machine learning model to predict *TROP2* gene expression. The graph illustrates performance of the predictive model assessed via Pearson's correlation in a leave-one-out cross-validation schema.

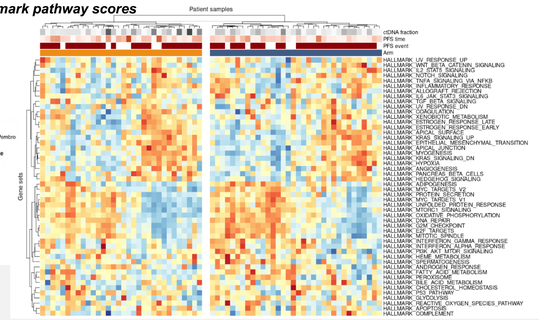
B Analysis similar to (A) stratifying patients (ctDNA ≥5%) into *TROP2*-high and *TROP2*-low groups using the top tertile of predicted *TROP2* expression as a threshold. *TROP2* expression was not significantly associated with PFS when combining treatment arms (HR = 1.08, P = 0.81) or in each individual arm (above). Current work is aimed at improving the *TROP2* expression model to re-examine potential associations with outcome.

Figure 5: Pathway analysis highlights clusters of similarity in tumor biology across the study population

A ***Pathway analysis workflow***



B ***Hallmark pathway scores***

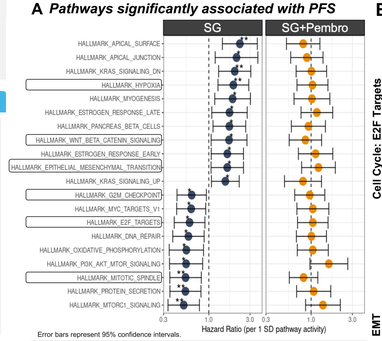


A Analysis workflow used to assess pathway activity scores per patient (≥3% ctDNA) from individual epigenomic features (promoters or enhancers) using GSVA[4].

B Heatmaps display GSVA-derived pathway activity scores in baseline plasma samples from the SG and SG+Pembro arms. Scores were derived using the HALLMARK gene set collection from MSigDb[5] using promoter signal and were converted to Z-scores to facilitate inter-pathway comparisons. Samples were clustered by treatment arm using Spearman correlation (1–ρ) as distance. **Both arms display similar heterogeneity in the activation of various pathways**: Cluster of patients have upregulated **hypoxia, estrogen and inflammatory signaling** while a distinct cluster of patients have upregulated **metabolic and cell cycle pathways**.

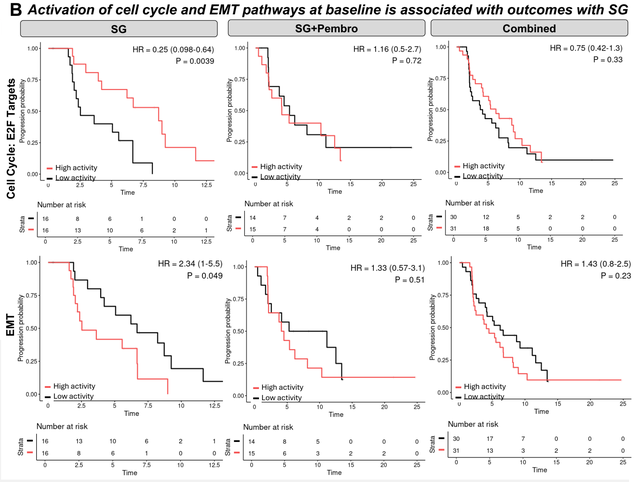
Figure 6: Interrogation of the plasma epigenome delineates pathways that drive sensitivity to SG

A ***Pathways significantly associated with PFS***



Error bars represent 95% confidence intervals.

B ***Activation of cell cycle and EMT pathways at baseline is associated with outcomes with SG***



A Pathways significantly associated with PFS across SG (N=34) and SG+Pembro (N=32) arms. HR and 95% CIs were generated from CoxPH models fitting PFS on the Z-score of pathway activity scores. FDRs are BH-corrected P-values from analysis of deviance tests comparing CoxPH models fit using pathway activities and log2(ctDNA fraction) to CoxPH models fit using only log2(ctDNA fraction). Only samples with ctDNA ≥3% were used for this analysis. *FDR≤10%, **FDR≤5%.

B Patients (≥5% ctDNA) in SG and SG+Pembro cohorts were classified into pathway-high (≥ median GSVA score) and pathway-low (< median GSVA score). CoxPH models were fit to evaluate the association between group assignment and PFS (HRs and P-values).

CONCLUSIONS

- This study demonstrates the feasibility of a comprehensive epigenomic liquid biopsy platform for non-invasive characterization of therapeutic response and resistance to SG with or without pembrolizumab in HR+/HER2- mBC.
- Baseline predicted *TROP2* expression was not significantly associated with PFS. Pathway analysis from the plasma epigenome suggests patients with high cell cycle or EMT activation may confer sensitivity or resistance to SG, respectively. Further analysis could enable identification of patients who may derive additional benefit from the addition of immunotherapy to SG.
- By providing real-time insight into transcriptional regulation, this approach may improve patient stratification, guide ADC treatment selection and inform rational drug combination strategies.

The authors would like to acknowledge Gilead Sciences and Merck Sharp & Dohme Corp. (subsidiary of Merck & Co., Inc., Rahway, NJ, USA) for providing drug and financial support for the trial, and Komen, METAvivor, Gateway, and the Mehlman Family Fund for providing financial support for the study.

1) Rugo et al., *J Clin Oncol*, 2022; 2) Baca et. al., *Nat Med*, 2023; 3) McLean et al., *Nat Biotechnol*, 2010; 4) Hänzelmann et. al., *BMC Bioinformatics*, 2013; 5) Liberzon et. al., *Cell Syst*, 2015.