

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

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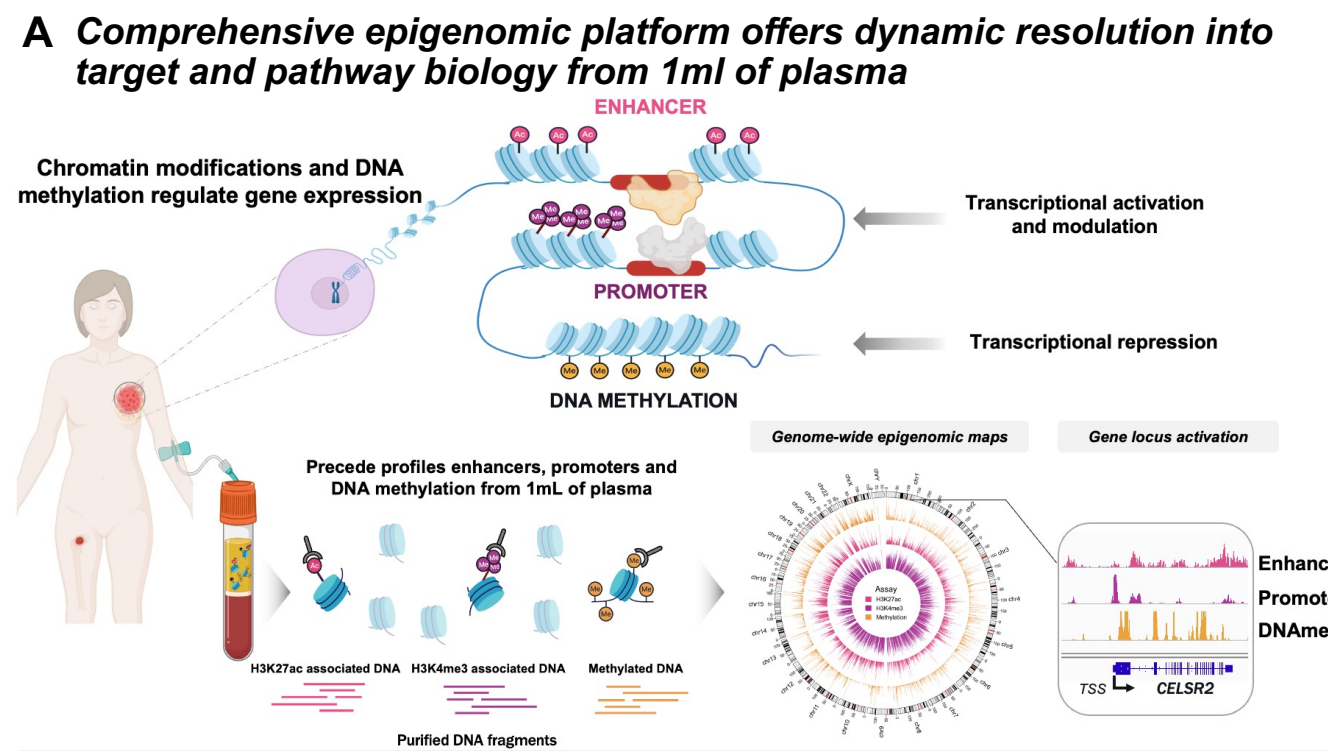
BACKGROUND

- 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

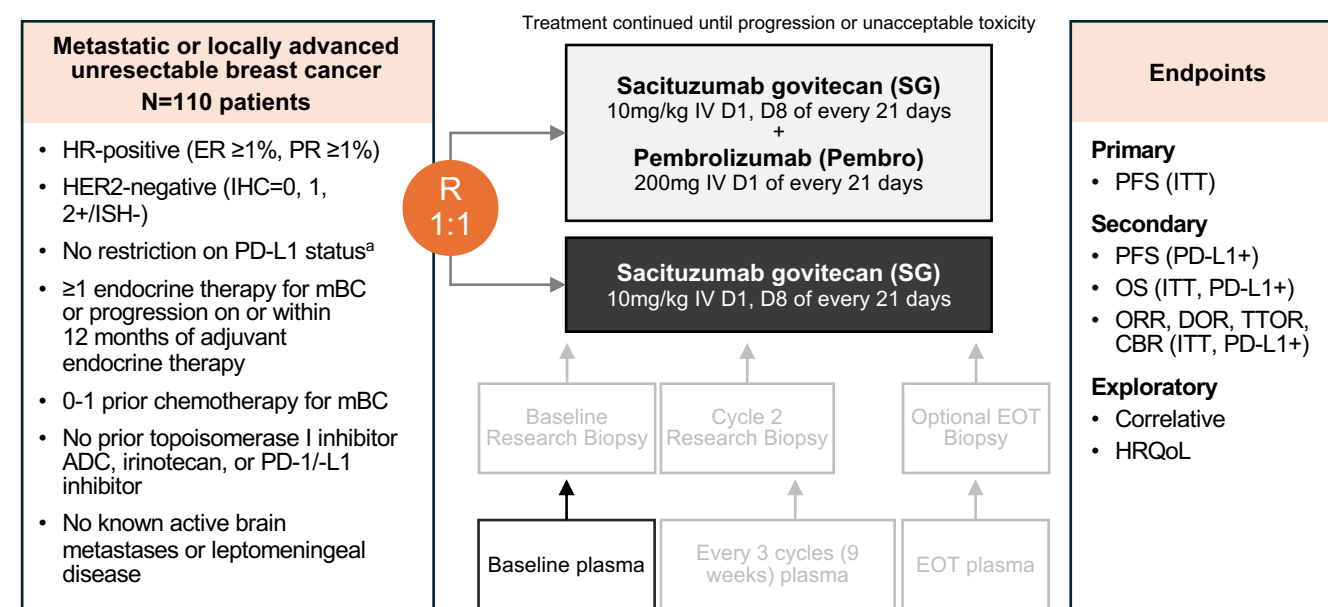
- Baseline plasma samples were collected from patients with HR+/HER2- mBC who enrolled in SACI-IO HR+.
- Genome-wide signals from promoters, enhancers and DNA methylation were profiled from 1mL of plasma from 95 patients, of which 80 patients met the assay quality control thresholds and ctDNA metrics required for downstream analysis (ctDNA $\geq 0.5\%$, 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.

Figure 1: Comprehensive epigenomic profiling from 1 mL 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 cells².

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			
$\geq 10\%$	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)

^aPatients diagnosed with de novo stage IV breast cancer excluded from denominator.

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

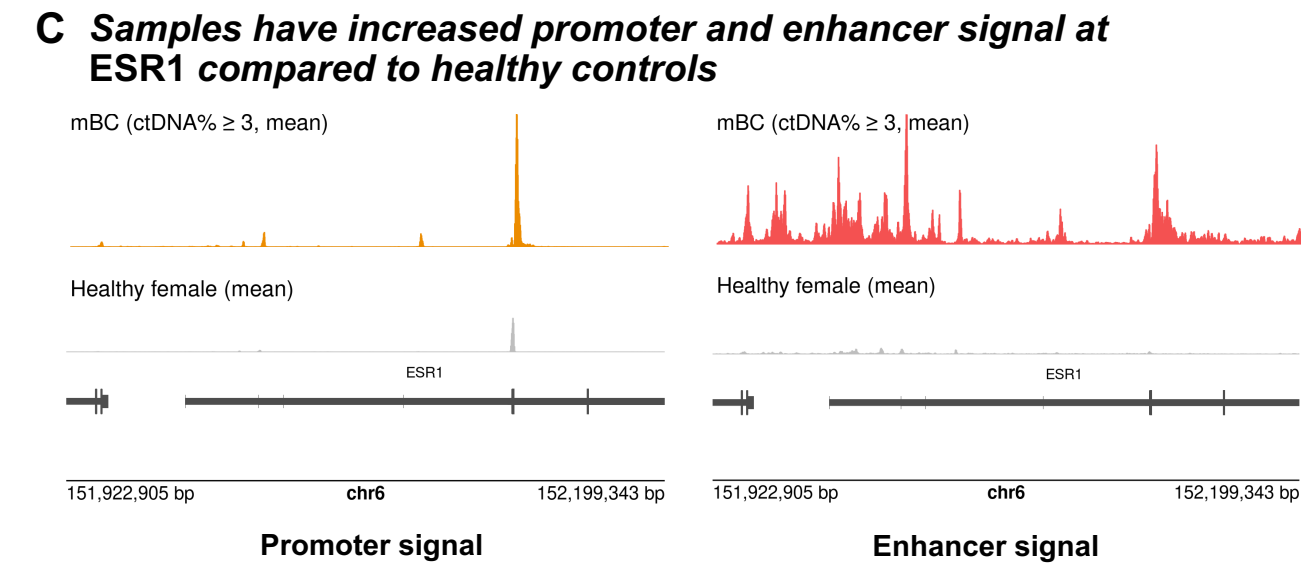
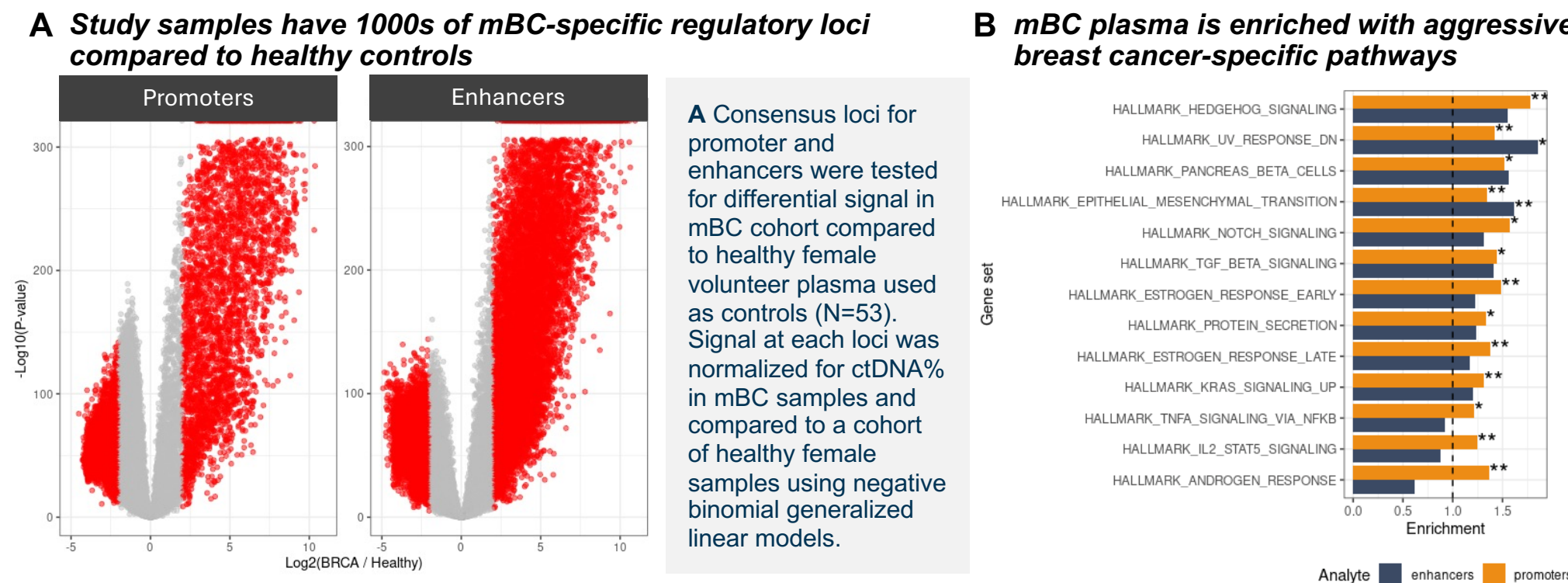


Figure 3: Higher baseline ctDNA fraction is associated with worse PFS

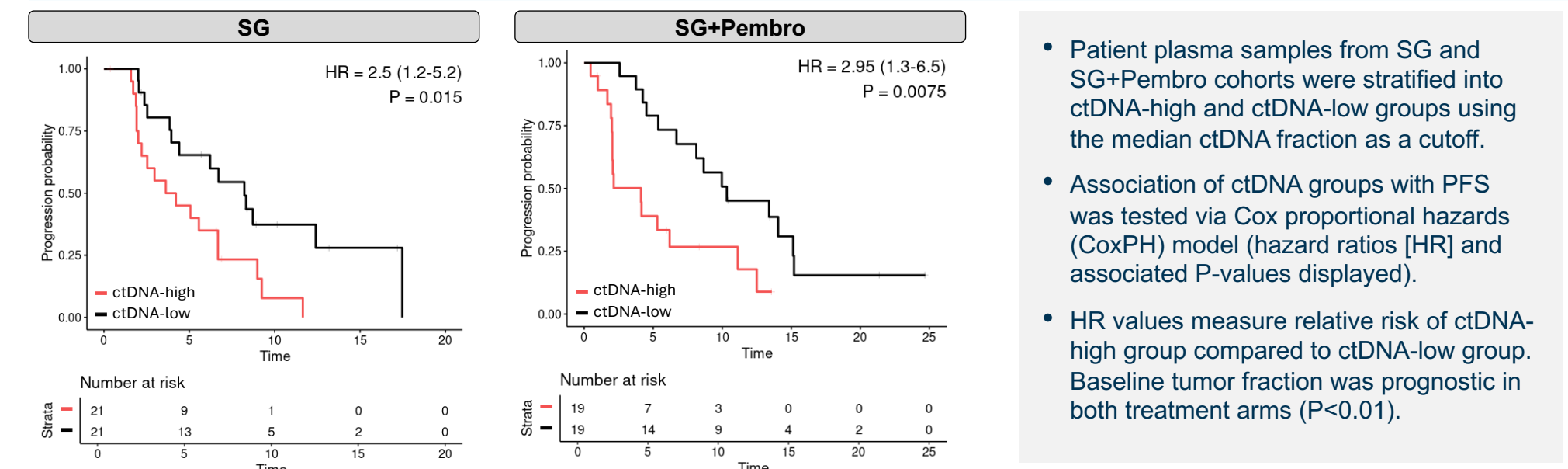


Figure 4: Higher baseline predicted TROP2 expression is not significantly associated with PFS

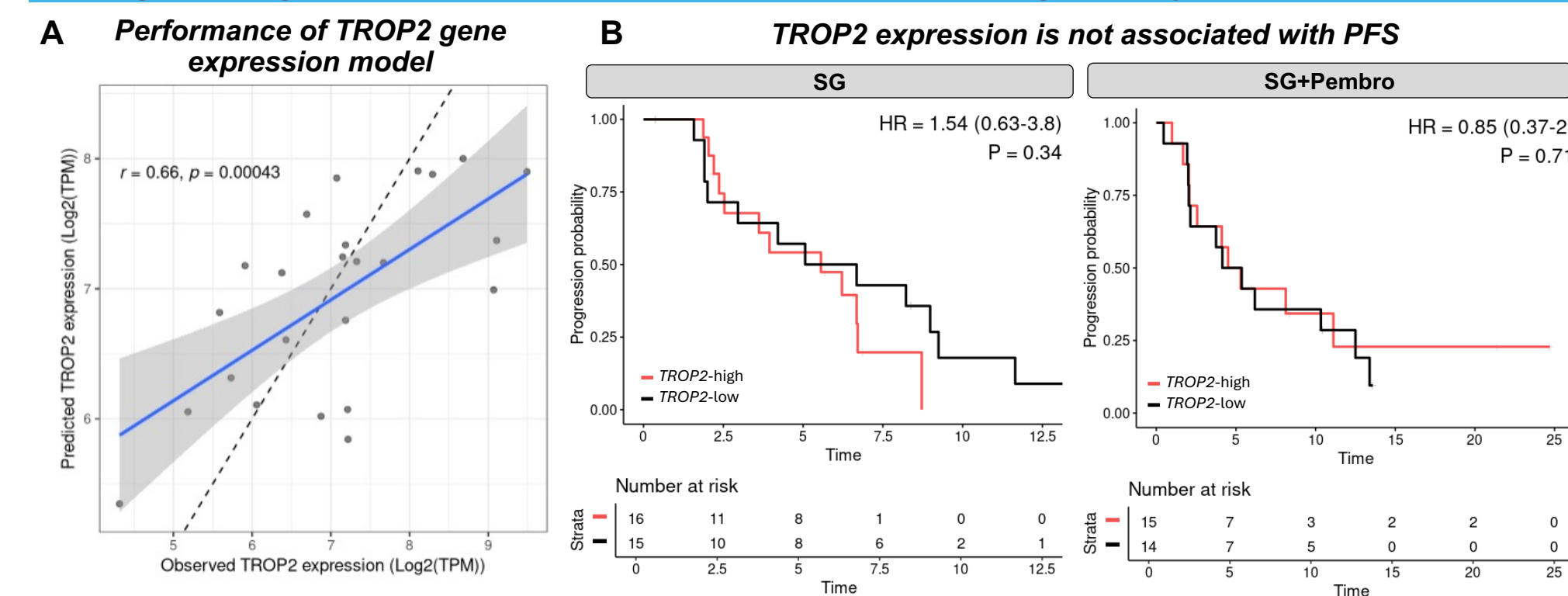


Figure 5: Pathway analysis highlights clusters of similarity in tumor biology across the study population

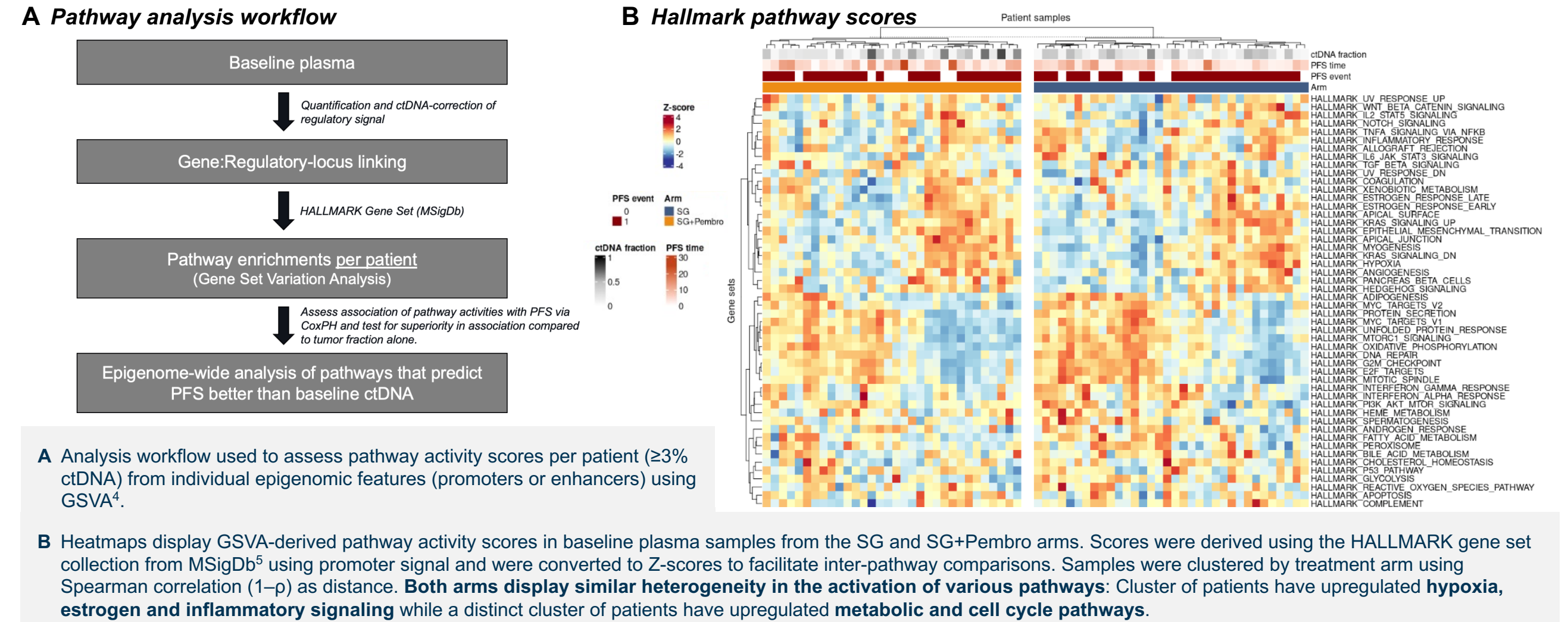
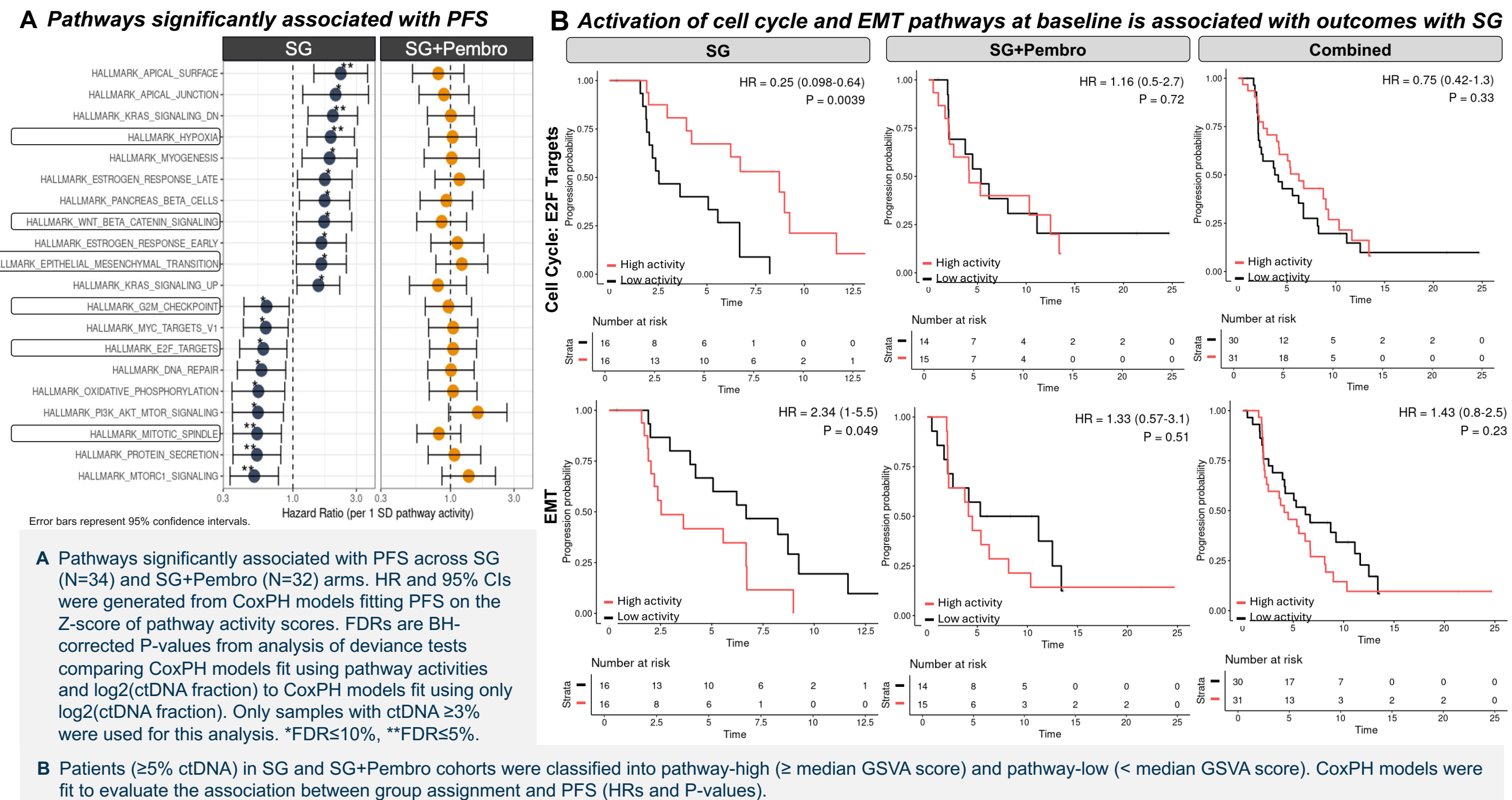


Figure 6: Interrogation of the plasma epigenome delineates pathways that drive sensitivity to SG



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.

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