Poster 26

Multi-trial evaluation of genome-wide targeted ctDNA methylation profiling for detection and classification of hepatobiliary cancers

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Objectives

1. Assay feasibility and detection: sensitivity for baseline cancer-signal detection across BTC and HCC in three phase 3 trials, and the clinical variables driving detection.
2. Cancer subtype classification: Cancer Signal of Origin calls from the Portrait classifier (liver-bileduct vs pancreas-gallbladder), and their limits for the hepatobiliary subtypes ICC, ECC and GBC.
3. Classifier refinement: whether a research-grade HCC-versus-adenocarcinoma classifier separates ICC from HCC.

Conclusions

1. Targeted methylation profiling detected cancer signal in 86–95% of hepatobiliary cancers, including early-stage disease (≥83% in well-differentiated BTC; ≥76% in BCLC A HCC).
2. The Portrait classifier assigned 94–95% of detected HCC to liver-bileduct but only partly resolved BTC subtypes: ICC was heterogeneous, and ECC and GBC were indistinguishable.
3. The research-grade classifier separated BTC from HCC (99.2% BTC accuracy; 77.4–81.9% HCC), with ICC as a distinct sub-cluster; 18–23% of confirmed HCC were adenocarcinoma-like, with lower tumor methylation fraction (p=0.0038).
4. Methylation-based separation of ICC from HCC could address a critical diagnostic gap and warrants further classifier refinement and validation.

Plain language summary

Why did we perform this research?
Liver cancer and bile duct cancer can look similar on scans but are treated very differently and telling them apart is difficult.

How did we perform this research?
We used a blood test that reads DNA methylation patterns from tumor DNA in blood, taken before treatment from 657 patients in three large trials.

What were the findings of this research?
The test found a cancer signal in 86–95% of samples, including earlier-stage disease, and told liver cancer apart from bile duct cancer.

What are the implications of this research?
A single blood test, with no tissue sample needed, may help doctors diagnose and classify liver and bile duct tumor that are hard to tell apart.

References

1. Desai M, et al. AACR 2023; LB297. 2. Liu MC, et al. Ann Oncol 2020;31:745. 3. Jamshidi A, et al. Cancer Cell 2022;40:1537. 4. Mellon CA, et al. Cancers 2023. 5. Kaseb AO, et al. Clin Cancer Res 2019. 6. AstraZeneca internal data (Oh DY, et al.).

Acknowledgements

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Disclosures & registration

ClinicalTrials.gov: TOPAZ-1 NCT03875235; HIMALAYA NCT03298451; EMERALD-1 NCT03778957. IRB-approved at all participating centres.

Poster

Supplementary material

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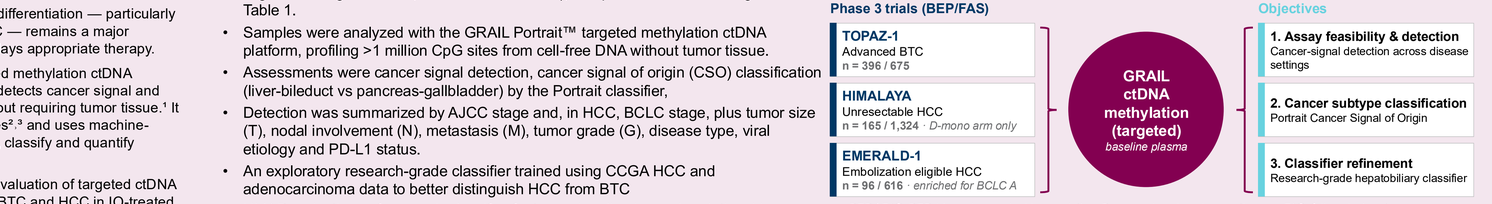
Introduction

- BTC (Intrahepatic cholangiocarcinoma, ICC; Extrahepatic cholangiocarcinoma, ECC; Gallbladder cancer, GBC) and HCC differ in management and prognosis yet can overlap clinically and radiographically.
- Non-invasive detection and differentiation — particularly distinguishing ICC from HCC — remains a major diagnostic challenge that delays appropriate therapy.
- GRAIL's tumor-naive targeted methylation ctDNA platform (GRAIL Portrait™) detects cancer signal and predicts tissue of origin without requiring tumor tissue.[1] It analyzes >1 million CpG sites[2,3] and uses machine-learning classifiers to detect, classify and quantify ctDNA.[4]
- First large-scale, multi-trial evaluation of targeted ctDNA methylation profiling across BTC and HCC in IO-treated populations (N=657 profiled).

Methods

- Baseline plasma from 657 patients across three independent global phase 3 trials of durvalumab-based regimens: TOPAZ-1 (1L advanced BTC), HIMALAYA (1L unresectable HCC, durvalumab-monotherapy arm only) and EMERALD-1 (TACE-eligible locoregional HCC, enriched for BCLC A); sample numbers are in Figure 1 and Table 1.
- Samples were analyzed with the GRAIL Portrait™ targeted methylation ctDNA platform, profiling >1 million CpG sites from cell-free DNA without tumor tissue.
- Assessments were cancer signal detection, cancer signal of origin (CSO) classification (liver-bileduct vs pancreas-gallbladder) by the Portrait classifier,
- Detection was summarized by AJCC stage and, in HCC, BCLC stage, plus tumor size (T), nodal involvement (N), metastasis (M), tumor grade (G), disease type, viral etiology and PD-L1 status.
- An exploratory research-grade classifier trained using CCGA HCC and adenocarcinoma data to better distinguish HCC from BTC
- UMAP projections of research-classifier features visualized sample relationships.

Figure 1. Three phase 3 trials, one targeted methylation assay, three objectives



n = BEP / FAS. BEP, biomarker-evaluable population; D-mono, durvalumab monotherapy; FAS, full analysis set.

Results and interpretation

1.a Assay feasibility and cancer-signal detection (Table 1)

- Cancer signal was detected in 95% of advanced BTC (TOPAZ-1), 94% of unresectable HCC (HIMALAYA) and 86% of TACE-eligible locoregional HCC (EMERALD-1).
- Detection exceeded that of previous mutation-based ctDNA datasets in comparable advanced disease for both BTC (95% TOPAZ-1 vs 82% benchmark[6]) and HCC (94% HIMALAYA vs 88% benchmark[5]).
- Performance was consistent across studies within the same disease stage, supporting use from locoregional therapy to first-line systemic treatment.

Figure 2. Baseline cancer-signal detection by clinical category and trial



Figure 2. Rows are trials, columns are clinical dimensions. Stacked bars give the percentage of QC-pass biomarker-evaluable samples per category (plum, cancer signal detected; turquoise, not detected; grey, not assessed); labels give n (%). Y-axis truncated to 60–100%. TOPAZ-1, BTC; HIMALAYA and EMERALD-1, HCC. BCLC stage, disease type and viral etiology are not applicable to all trials; EMERALD-1 N and M were not available in the clinical dataset. AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer.

Table 1. Cancer-signal detection by study

Study	Indication	Disease setting	N (BEP)	Cancer detected
TOPAZ-1	Advanced BTC	1L systemic	396	375 (95%)
HIMALAYA	Unresectable HCC	1L systemic	165	155 (94%)
EMERALD-1	Locoregional HCC	TACE-eligible	96	83 (86%)

BEP, biomarker-evaluable population; BTC, biliary tract cancer; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization.

1.b Detection across disease stage and clinical category (Figure 2, right)

- BTC (TOPAZ-1): strong AJCC-stage gradient (I 85% → II 86% → III 90% → IV 98%; p=0.0003), stepping up sharply at stage IV. Metastasis (~88% non-metastatic vs ~98% metastatic), tumor grade (~84% well-differentiated vs ~96% moderate/poor) and disease type (~85% recurrent vs ~98% initially unresectable) were additional drivers.
- HCC (HIMALAYA, EMERALD-1): gradients across BCLC stage (A → B, then plateau), AJCC stage (EMERALD-1 I 75.9% → III 95.5%; HIMALAYA already at ceiling, I–II 100%, III–IV ~92–93%) and tumor size (T1 → T2+); detection was lower in non-viral ("Other") etiology.
- Detection did not differ by nodal involvement or PD-L1 status in either disease (all p>0.05).
- Clinically meaningful sensitivity was maintained at the low end of every gradient (≥83% in well-differentiated BTC; ≥76% in BCLC A HCC).

2. Cancer subtype classification — CSO (Table 2 and Figure 3)

- BTC subtypes were only partly resolved: ECC and GBC were predominantly pancreas-gallbladder (~79% each) and indistinguishable, while ICC split between liver-bileduct (53%) and pancreas-gallbladder (33%).
- Portrait CSO calls were highly concordant for HCC: 94–95% of detected HIMALAYA and EMERALD-1 samples were liver-bileduct.
- But CSO did not reliably resolve anatomic BTC subtypes — the motivation for objective 3.

Figure 3. Anatomic origins of the BTC subtypes

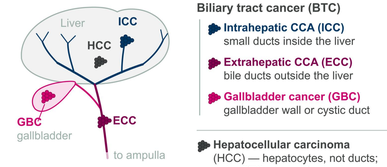


Table 2. Cancer Signal of Origin (CSO) prediction

Study	Indication	Samples detected	Liver-bileduct	Pancreas-gallbladder	Other (GI)	Other (non-GI)
TOPAZ-1	Advanced BTC	375/396 (94.7%)	119 (31.7%)	203 (54.1%)	26 (6.9%)	27 (7.2%)
	ICC	204/214 (95.3%)	109 (53.4%)	68 (33.3%)	13 (6.4%)	14 (6.9%)
	ECC	75/83 (90.4%)	5 (6.7%)	59 (78.7%)	6 (8.0%)	5 (6.7%)
	GBC	96/99 (97.0%)	5 (5.2%)	76 (79.2%)	7 (7.3%)	8 (8.3%)
HIMALAYA	Unresectable HCC	155/165 (93.9%)	147 (94.8%)	0 (0%)	2 (1.3%)	6 (3.9%)
EMERALD-1	Embolization eligible HCC	83/96 (86.5%)	78 (93.9%)	1 (1.2%)	0 (0%)	4 (4.8%)

ICC heterogeneity reflects known clinical diagnostic challenges; ECC and GBC are indistinguishable by the Portrait classifier, and ~14% of BTC samples were classified as other origins. Concordance for HCC was strong (94–95% liver-bileduct). Percentages are of samples with cancer signal detected. ECC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; GI, gastrointestinal; ICC, intrahepatic cholangiocarcinoma.

3. Classifier refinement — research-grade hepatobiliary classifier (Table 3 and Figures 4–5, right)

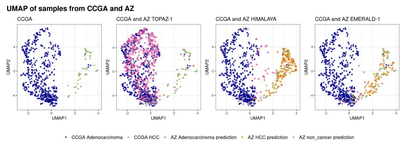
- Successfully identified 99.2% of BTC as adenocarcinoma-like: only 3 of 375 samples were called HCC-like.
- About (77-82%) of HCC correctly classified; HIMALAYA (77.4%) and EMERALD-1 (81.9%)
- 18–23% of confirmed HCC were called adenocarcinoma-like. These samples had lower tumor methylation fraction (mVAF) than HCC-like samples (36% vs 60% mVAF-high; Fisher p=0.0038), which may give suboptimal signal for accurate classification.

Table 3. Research-grade classifier: HCC-like vs adenocarcinoma-like calls

Study	Detected (Portrait)	HCC-like	Adenocarcinoma-like
TOPAZ-1 (BTC)	375	3 (0.8%)	372 (99.2%)
HIMALAYA (HCC)	155	120 (77.4%)	35 (22.6%)
EMERALD-1 (HCC)	83	68 (81.9%)	15 (18.1%)

Research-grade classifier developed by GRAIL from pre-treatment CCGA (Circulating Cell-free Genome Atlas) data, trained on HCC versus adenocarcinoma samples (including ICC, extrahepatic bile duct cancer, gallbladder cancer and adenocarcinomas from other organ sites). For every sample with cancer detected by the Portrait pipeline the classifier returns one of two calls: hepatocellular carcinoma or adenocarcinoma.

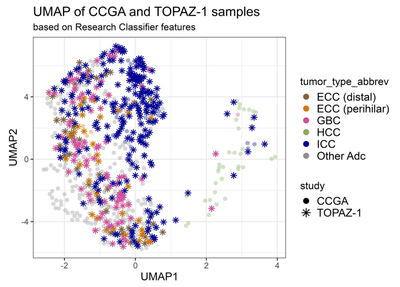
Figure 4. Confirmation of BTC–HCC separation in methylation space



HCC and adenocarcinoma form distinct clusters in methylation space. TOPAZ-1 BTC samples overlay within the adenocarcinoma region and HIMALAYA and EMERALD-1 HCC samples overlay within the HCC region, showing clear separation. The adenocarcinoma-like HCC samples do not cluster with true adenocarcinoma in methylation space; whether this reflects a technically challenging low-signal scenario, a biologically distinct HCC subset, or both, requires further investigation.

CCGA, Circulating Cell-free Genome Atlas; mVAF, median variant allele frequency (tumor methylation fraction); UMAP, uniform manifold approximation and projection.

Figure 5. Resolution of ICC from HCC by the research-grade classifier



ICC forms a distinct sub-cluster separable from HCC; GBC and ECC overlap substantially with each other, explaining why the Portrait classifier cannot distinguish them.