



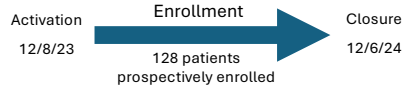
Cancer signal origin (CSO) prediction in cancer of unknown primary (CUP) syndrome (CRONUS) using circulating tumor DNA (ctDNA)

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Background

- Determining the site of origin remains a difficult problem for patients with CUP.
- Use of empiric chemotherapy is associated with variable responses and poor survival outcomes.
- Emerging data indicate site-specific therapy tailored to tissue-of-origin predicted by molecular (gene-expression) profiling may improve outcomes in CUP.
- However, high assay failure rates (30-40%) from lack of sufficient tissue can limit clinical and research utility.
- A blood (ctDNA) based methylation assay with CSO capabilities circumvents tissue scarcity and can guide site-specific therapy.

Methods



- Blood (N = 125) was tested (N = 123; 2 hemolyzed) with a targeted ctDNA methylation panel.
 - Cohort A, treatment naïve patients = 56
 - Cohort B, previously treated patients = 67
- Primary endpoint was feasibility:** proportion of CUP patients with completed ctDNA testing with CSO returned.
- Key secondary endpoint was concordance:** agreement between assay CSO and putative primary sites predicted by consensus clinicopathological review by 2 independent investigators and a CUP multidisciplinary team.

CONSORT Diagram

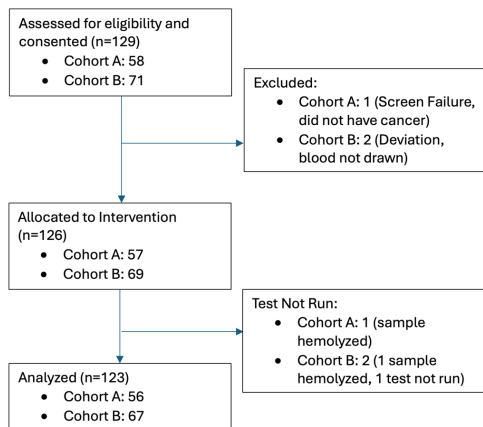


Table 1 – Patient Characteristics

Variable	Total	%	Cohort A	%	Cohort B	%	P-value
	N = 123		N = 56		N = 67		
Age (years)	Median 66		69		64		0.19
	Range 27-88		29-88		27-88		
Gender	Male 68	55.3	25	44.6	43	64.2	0.045
	Female 55	44.7	31	55.4	24	35.8	
ECOG PS	0 44	35.8	23	41.1	21	31.3	0.76
	1 61	49.6	23	41.1	38	56.7	
	≥ 2 18	14.6	10	17.9	8	11.9	
Histology	Adenocarcinoma 56	45.5	19	33.9	37	55.2	0.06
	Carcinoma 48	39.0	26	46.4	22	32.8	
	Malignant Neoplasm 12	9.8	8	14.3	4	6.0	
	Squamous Cell 7	5.7	3	5.4	4	6.0	
NLR	Low (< 5) 66	53.7	33	58.9	33	49.3	0.55
	High (≥ 5) 39	31.7	22	39.3	17	25.4	
	Unknown 18	14.6	1	1.8	17	25.4	
NMS	< 3 85	69.1	35	62.5	50	74.6	0.17
	≥ 3 38	30.9	21	37.5	17	25.4	
Liver Metastases	No 83	67.5	33	58.9	50	74.6	0.08
	Yes 40	32.5	23	41.1	17	25.4	

Figure 1 – CSO Assay vs Clinician Prediction

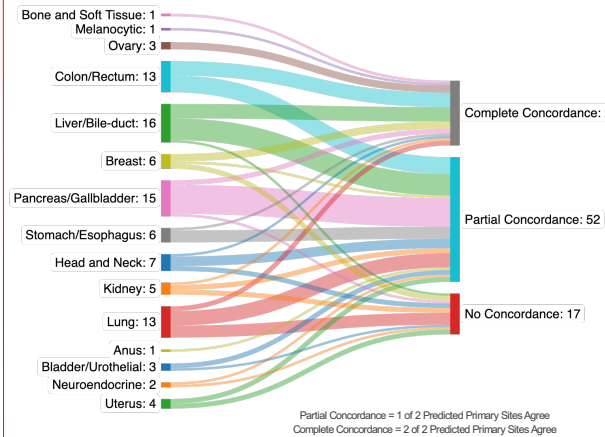
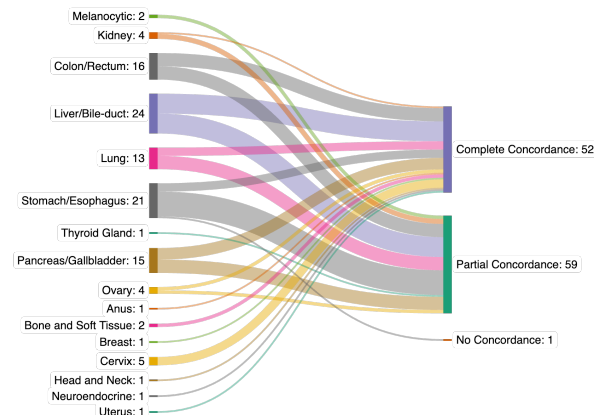


Figure 3 – Inter-Clinician Variability



Key Findings

Feasibility:
 78% return rate

Cohort A: 79%; Cohort B: 78%

Concordance:
 82% between assay and clinicians
 Complete: 28%; Partial: 54%

Figure 2 – Concordance of CSO Assay vs Clinicians

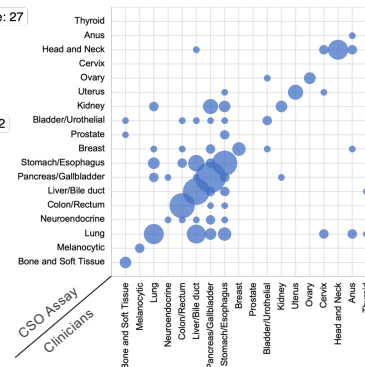


Figure 4 – Tumor Methylation Fraction (TMEF)

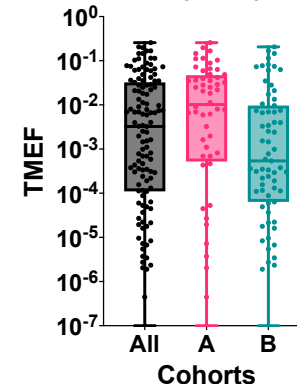


Table 2 – Discordant Predictions

ID	Cohort	Clinician 1	Clinician 2	CSO 1	CSO 2	Overlap?
5	A	Stomach/Esophagus	Liver/Bile duct	Lung	Neuroendocrine	
6	A	Anus	Colon/Rectum	Head and Neck	Bladder/Urothelial	Yes
16	B	Stomach/Esophagus	Pancreas/Gallbladder	Kidney	Liver/Bile duct	Yes
23	A	Pancreas/Gallbladder	Stomach/Esophagus	Breast	Prostate	
28	B	Bladder/Urothelial	Other	Breast	Prostate	
32	A	Stomach/Esophagus	Liver/Bile duct	Pancreas/Gallbladder	Lung	Yes
33	A	Liver/Bile duct	Pancreas/Gallbladder	Bladder/Urothelial	Breast	
34	B	Cervix	Anus	Head and Neck	Lung	Yes
49	A	Liver/Bile duct	Stomach/Esophagus	Lung	Kidney	
50	A	Stomach/Esophagus	Pancreas/Gallbladder	Liver/Bile-duct	Lung	Yes
61	A	Cervix	Bladder/Urothelial	Uterus	Ovary	Yes
87	A	Stomach/Esophagus	Pancreas/Gallbladder	Uterus	Lung	
89	A	Stomach/Esophagus	Pancreas/Gallbladder	Neuroendocrine	Bladder/Urothelial	
109	A	Cervix	Anus	Lung	Head and Neck	Yes
119	A	Cervix	Pancreas/Gallbladder	Lung	Neuroendocrine	
120	B	Thyroid Gland	Kidney	Lung	Pancreas/Gallbladder	
125	B	Lung	Bone and Soft Tissue	Kidney	Prostate	

*Overlap = clinical similarity between CSO Assay Predictions and Clinician Predictions

Conclusions

- The use of a blood-based ctDNA methylation assay to detect a putative primary site for patients with CUP is feasible.
- The assay's predicted CSO demonstrated high concordance with putative primary calls based on a comprehensive clinicopathologic work-up.
- Many predictions with no concordance had a related clinical picture to a CSO Assay prediction that may have appropriately guided therapy.
- MD Anderson Cancer Center's CUP Clinic can accrue patients with this rare malignancy to clinical trials in a robust fashion.
- This blood-based assay has the potential to fill in gaps in the diagnostic work-up focusing on the tissue of origin for patients with CUP.
- Further investigation with this ctDNA methylation assay can help reinvigorate the elusive search for a putative primary and thus tailored management of patients with this rare disease.

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