Real-world impact of HelioLiver Dx – a blood-based test for the early detection of hepatocellular carcinoma

HBV Forum 2025 – Session III: HCC

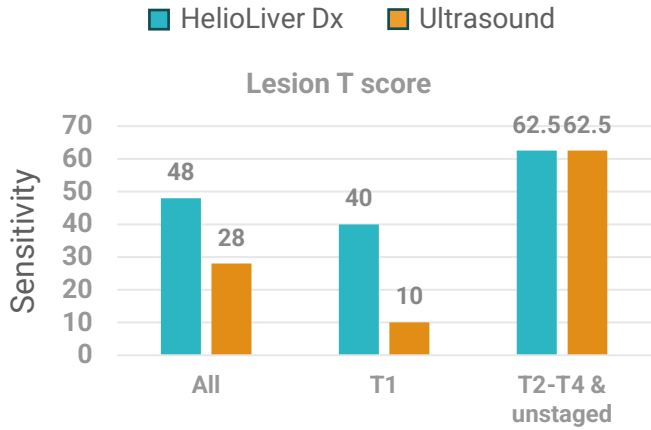
November 6, 2025

An overview of HelioLiver and results from prospective validation

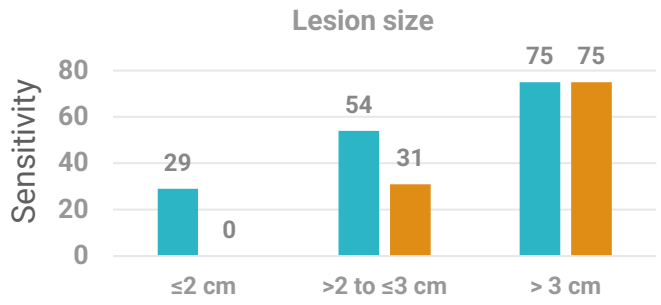
- **Cell-free DNA or cfDNA** is released into the bloodstream by dying cells, including dying tumor cells. cfDNA fragments arising from dying tumor cells carry **tumor associated aberrant methylation patterns.**
- cfDNA methylation assay: **~ 14000 CpG sites, over >1000 genes**
- Immunoassay: **3 protein biomarkers** that may be elevated in HCC.
- Helio's proprietary **AI/machine learning algorithm** combines multi-omic signals to produce a qualitative test result.
- A positive test result may indicate the presence of HCC. To be followed up by diagnostic imaging via MRI/CT

CLiMB : Prospective, multi-center, blinded, US-based clinical trial to validate and compare the performance of HelioLiver Dx to Ultrasound

- HelioLiver Dx outperformed ultrasound when it matters most – early stage and <3 cm lesions
- Passed primary, secondary and exploratory study endpoints



HelioLiver Dx is **4x more sensitive** than ultrasound for detecting T1 lesions



Helioliver Dx **detects more lesions <3cm** than ultrasound.

Ultrasound failed to detect any lesions ≤ 2cm

HelioLiver LDT

HelioLiver Case studies prepared by Dr. Rahul Maheshwari and Dr. Hrishikesh Samant

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HelioLiver is already transforming patient lives and clinical outcomes

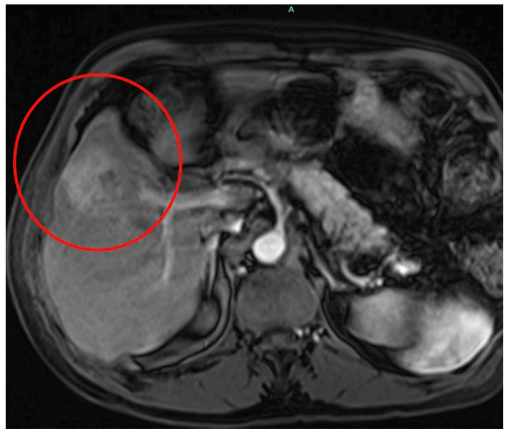
Now **commercially available** as a **Lab Developed Test (LDT)**, HelioLiver is being adopted in real-world clinical settings where its use has led to earlier detection and improved patient outcomes.

Real-world cases consistently demonstrate HelioLiver's superior performance compared to many routine testing modalities, reinforcing its potential to become the new standard of care in liver cancer screening and surveillance.

HelioLiver in action – real patients, real impact

Case #1:

63-year-old man with hepatitis B–related cirrhosis, asymptomatic, referred for evaluation of a newly detected liver lesion. MRI revealed a 3.5 cm segment 5/8 mass demonstrating arterial enhancement and central delayed enhancement without washout, classified as LR-3.



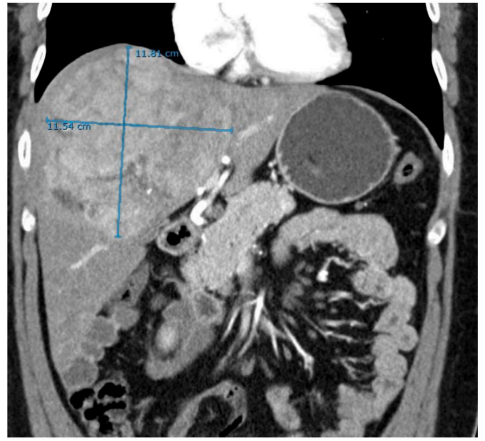
HelioLiver Result: Abnormal/Positive

Test	Value
AFP	**12.7** ng/mL (ref range 0.3 – 12.1 ng/mL)
AFP-L3%	**18.1** % (ref range <10 %)
DCP	Normal (ref range < 7.7 ng/mL)
Methylation score	**0.86** (ref range < 0.57)

- Liver mass biopsy was prompted by an abnormal HelioLiver result and revealed moderately differentiated HCC.
- Without a positive HelioLiver result, management would likely have involved serial imaging every 3 months, allowing further tumor growth in the interim.
- The patient subsequently underwent successful Y-90 radioembolization and is now awaiting liver transplant.

Case #2:

34-year-old male presenting with abdominal pain.
CT imaging revealed an 11.8 × 11.5 × 9.9 cm mass at the junction of the right and left hepatic lobes.
Lesion described as a heterogeneous mass with microscopic fat and showed a faint pseudocapsule with equivocal washout.



HelioLiver Result: Normal/Negative

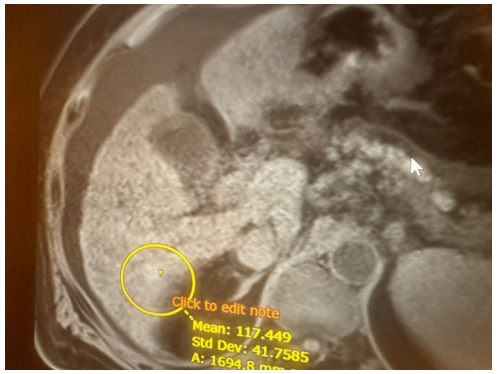
Test	Value
AFP	Normal (ref range 0.3 – 12.1 ng/mL)
AFP-L3%	Normal (ref range <10 %)
DCP	Normal (ref range < 7.7 ng/mL)
Methylation score	**0.37 Normal** (ref range < 0.57)

- Normal HelioLiver result -> prompted biopsy prior to any complex surgical resection due to the mass location.
  Multiple core biopsies showed benign liver tissue with no malignant cells.
  Follow-up imaging at 6 months demonstrated no change in size, further supporting a benign diagnosis.
  The negative HelioLiver result ultimately helped avoid a major surgery and its associated risks for what proved to be a non-malignant lesion.

HelioLiver in action – real patients, real impact

Case #3:

59-year-old male with MASLD-related cirrhosis (diagnosed 2020; MELD 13). Comorbidities: diabetes, obesity, COPD.
No decompensation and hepatic function is within normal limits.
Ultrasound surveillance over 4 years revealed no liver lesions.



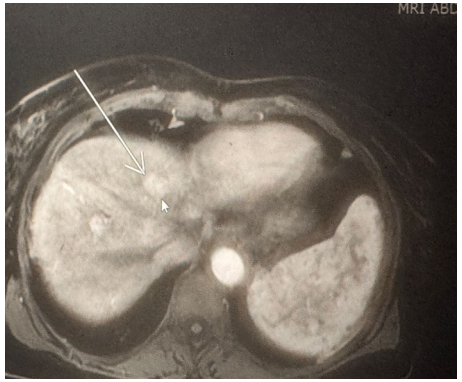
HelioLiver Result: Abnormal/Positive

Test	Value
AFP	**12.2** ng/mL (ref range 0.3 – 12.1 ng/mL)
AFP-L3%	**16.2** % (ref range <10 %)
DCP	**80.6** ng/mL (ref range < 7.7 ng/mL)
Methylation score	**0.85** (ref range < 0.57)

- MRI performed due to abnormal HelioLiver result identified an 8 mm lesion, classified as LI-RADS 3
- The lesion is suspected to represent an early-stage hepatocellular carcinoma (HCC) but does not yet meet full radiographic criteria for definite diagnosis.
- The management plan is to perform repeat MRI every 3 months.

Case #4:

77-year-old female diagnosed with cirrhosis (decompensated)
Ascites: Managed with lasix 40 mg and spironolactone 100 mg daily
EGD: grade 2 varices
Ultrasound detected no hepatic lesions



HelioLiver Result: Abnormal/Positive

Test	Value
AFP	**14** (ref range 0.3 – 12.1 ng/mL)
AFP-L3%	**18.1** (ref range <10 %)
DCP	**78** (ref range < 7.7 ng/mL)
Methylation score	**0.95** (ref range < 0.57)

- MRI performed due to abnormal HelioLiver result revealed a 19 mm lesion with arterial phase enhancement in left hepatic lobe. Compared with imaging from one year prior, the lesion has enlarged, meeting threshold growth criteria. Previous scan showed cirrhosis with a hyper vascular focus in the same region.
- Not a transplant candidate due to advanced age.
- Plan to proceed with Y-90 therapy given interval growth and positive methylation score. Patient has stable liver function and can tolerate locoregional treatment.

HelioLiver in action – real patients, real impact

Case #5:

Overview

- Patient: High-risk for HCC (hepatocellular carcinoma)
- **HelioLiver Result: Abnormal**
- Initial MRI: Negative - no focal lesion detected
- Follow-up Imaging: 8 mm lesion identified on subsequent scans

Key Findings

- The initial MRI failed to detect the early-stage tumor, likely due to its small size and atypical vascular pattern
- HelioLiver identified biological signals, cfDNA methylation and protein markers, before the tumor became radiographically visible
- Suggests that some HelioLiver "false positives" may, in fact, represent MRI false negatives, revealing disease at a biologically earlier stage.

HelioLiver: Enhancing Clinical Decision-Making and Early Detection

Both positive and negative results provide actionable insights for better patient management.

- Positive: Prompts timely follow-up (targeted imaging or biopsy) → earlier detection and treatment
- Negative: Offers clinical reassurance, avoiding unnecessary procedures and focusing on higher-risk patients

MRI remains the gold standard, but has limitations:

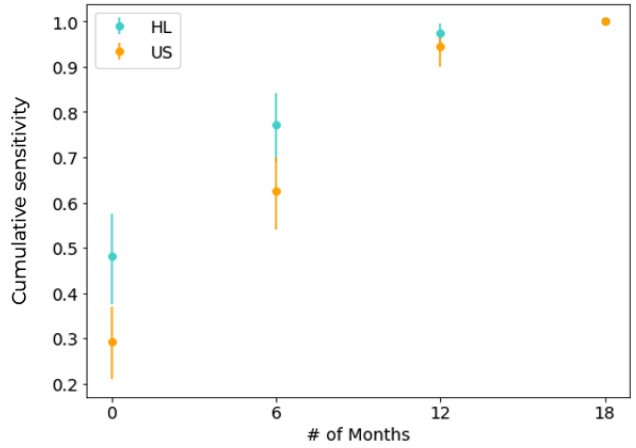
- May miss very small or atypical lesions (<1 cm) not yet visible
- Performance varies with scanner quality, reader expertise, and patient factors (iron overload, contrast use, motion)

HelioLiver Dx detects biological signals of HCC that can appear before imaging abnormalities, offering a complementary, earlier window of detection and supporting more confident clinical decisions.

HelioLiver Dx outperforms ultrasound in detecting early-stage lesions in a surveillance setting

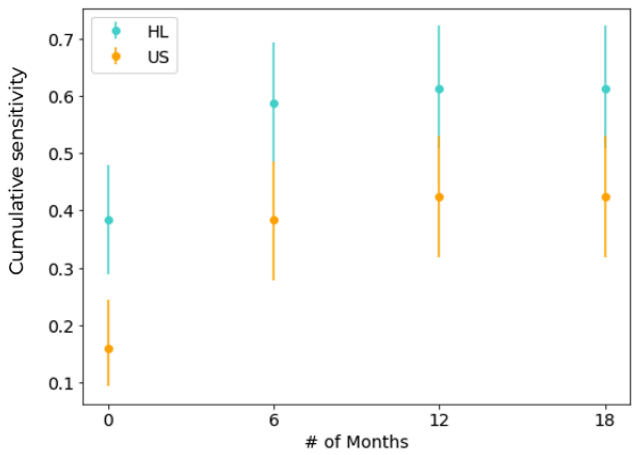
- CLiMB study was cross-sectional in nature and longitudinal test performance was not reported.
- Monte Carlo simulations were performed to compare the expected performance of ultrasound and HelioLiver Dx over time), assuming an adherence rate of 100% and a tumor doubling time of 6 months.
- **Results demonstrate that HelioLiver Dx detects HCC earlier than ultrasound.**

1A. Cumulative Sensitivity (t0, t1, t2, t3) for all HCC



Modality	t0	t1=6mo	t2=12mo	t3=18mo
HELIOLIVER DX	0.482	0.772	0.976	1
ULTRASOUND	0.292	0.626	0.945	1

1B. Cumulative Sensitivity (t0, t1, t2, t3) for Early HCC



Modality	t0	t1=6mo	t2=12mo	t3=18mo
HELIOLIVER DX	0.384	0.587	0.612	0.612
ULTRASOUND	0.159	0.383	0.423	0.423

1C. Tumor size at diagnosis of HCCs detected by 18 months

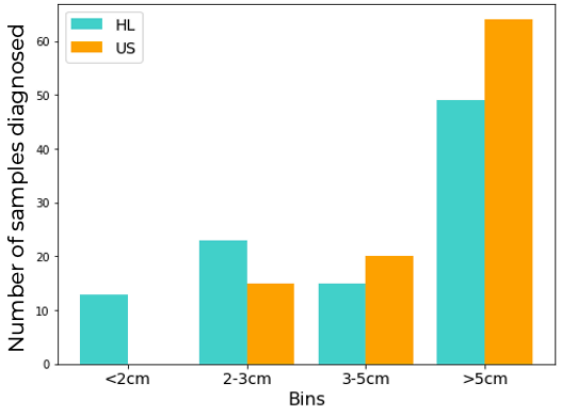


Figure 1. Baseline case assuming 100% adherence for both HelioLiver Dx (HL) and Ultrasound (US).

A) Cumulative sensitivities at t0, t1=6months, t2=12 months and t3=18 months. By 18 months, all HCCs were diagnosed by both modalities.

B) Cumulative sensitivities at each test time point for early-stage lesions. For each timepoint, sensitivity was calculated as the number of early-stage tumors detected divided by total number of early-stage tumors at the first timepoint.

C) Size distribution for when HCCs were diagnosed by each modality. HL detects tumors earlier than US.

HelioLiver Dx is more cost-effective than ultrasound

Check out our poster of distinction at AASLD 2025!

Abstract #3132 | "A Markov Model for Cost-Effectiveness Analysis Comparing the Novel Blood-Based HELIOLIVER® Test vs. Liver Ultrasound Semiannually for Early Detection of Hepatocellular Carcinoma" demonstrates how HELIOLIVER® is transforming liver health by making screening more accessible than traditional imaging.

Study led by:
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Oncoguard Liver

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Oncoguard Liver: A *Blood-based* Test for HCC Detection

In vitro diagnostic assay to analyze patients' **blood** for the presence of **Methylated DNA Markers** (MDMs) and a **protein biomarker**—yielding qualitative results

Methylated DNA Markers + **Alpha-fetoprotein (AFP)** + **Patient Sex**

HOXA1, *TSPLY5*, *B3GALT6* (reference)

Intended Patient Population

The mt-HBT test is indicated as an aid in the detection of HCC for adults with liver cirrhosis and/or chronic hepatitis B (HBV) who are at an increased risk for HCC.

B3GALT6: beta-1,3-galactosyltransferase 6, cfDNA: cell-free DNA, HCC: Hepatocellular carcinoma, *HOXA1*: homeobox A1, mt-HBT: multi-target-Hepatocellular carcinoma Blood Test, *TSPYL5*: Testis specific protein Y-linked like 5

Chalasani NP, et al. *Clin Gastroenterol Hepatol*. 2022;20:173-182.

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Oncoguard Liver Summary

Marker Discovery[1]

- Over 300 differentially methylated regions identified from frozen tissue
- Technical and biological validation narrowed candidates
- Phase I and Phase II plasma studies

Marker Selection[2]

- Assess performance of candidate
- Achieved high sensitivity for early-stage HCC and all-stage HCC
- 135 HCC cases and 302 controls

Algorithm Development[3]

- Finalize biomarker panel and develop algorithm
- 82% overall, 71% early-stage HCC sensitivity at 88% specificity
- 136 cases and 404 controls training set

Assay Clinical Lab Validation[3]

- Validate performance in independent cohort
- 88% overall, 82% early-stage HCC sensitivity at 87% specificity
- 156 cases and 245 controls

ALTUS STUDY

2019 — 2025

mt-HBT: multi-target Hepatocellular carcinoma Blood Test, HCC: Hepatocellular carcinoma

1. Kisiel JB, et al. *Hepatol*. 2019;69(3):1180-1192. 2. Chalasani NP, et al. *Clin Gastroenterol Hepatol*. 2021;19:2597-2605. 3. Chalasani NP, et al. *Clin Gastroenterol Hepatol*. 2022:20:173-182.

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The ALTUS Study (Alternative to Ultrasound)

- Prospective, multicenter study in a surveillance population in the United States (NCT05064553).
- Evaluated mt-HBT performance with a head-to-head comparison to standard-of-care ultrasound for early-stage HCC sensitivity.
- Ultrasound surveillance followed by contrast-enhanced CT/MRI as reference standard.

EXACT SCIENCES
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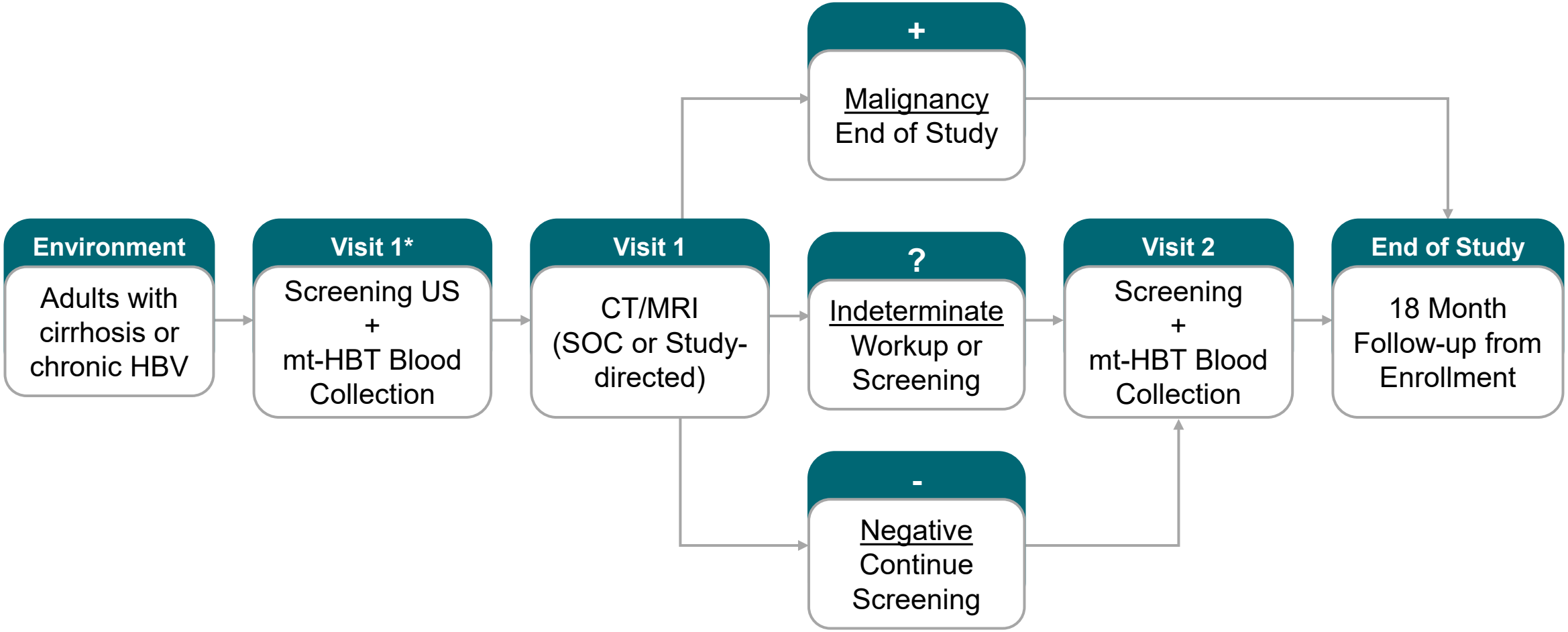
ALTUS Endpoints

- **Primary:**
  - Non-inferiority of mt-HBT to ultrasound for early-stage HCC sensitivity* within a 5% margin, with sequential testing of superiority.
  - mt-HBT specificity >82%.
    - A 95% confidence interval lower bound of 82% was established as a clinically desirable target.
- **Secondary:**
  - mt-HBT overall HCC sensitivity

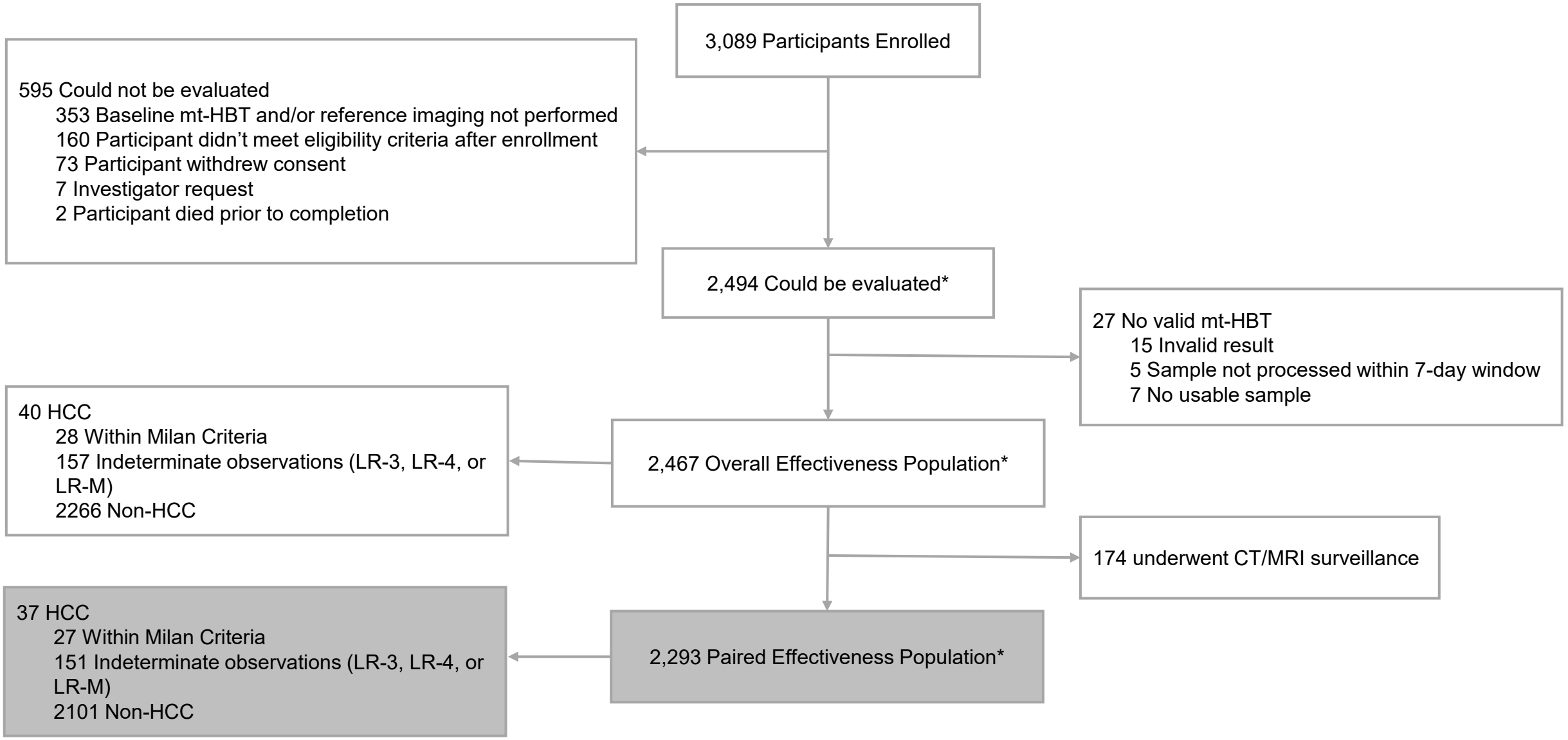
*Defined by Milan Criteria: Single lesion ≤5 cm or; up to 3 lesions, each ≤3 cm; no macrovascular invasion or extrahepatic metastases.
HCC, hepatocellular carcinoma; mt-HBT, multi-target HCC blood test

Study Design



CT/MRI, computed tomography/magnetic resonance imaging; HBV, hepatitis B virus; mt-HBT, multi-target HCC blood test.
*Imaging and blood collection were completed within 30 days.

Participant Enrollment and Disposition



Baseline Demographic Characteristics

Characteristics	Overall Effectiveness (N=2,467), n (%)	Paired Effectiveness (N=2,293), n (%)
Age, median (IQR)	64 (58-70)	64 (58-70)
Male	1,411 (57.2)	1,300 (56.7)
Race/Ethnicity		
White	1,946 (78.9)	1,807 (78.8)
Black	244 (9.9)	232 (10.1)
Asian	173 (7.0)	158 (6.9)
Hispanic or Latino	505 (20.5)	485 (21.2)
Cirrhosis etiologies	2,297 (93.1)	2,138 (93.2)
MASLD	1,000 (40.5)	954 (41.6)
ALD	670 (27.2)	606 (26.4)
HCV	346 (14.0)	320 (14.0)
HBV	55 (2.2)	52 (2.3)
Other	226 (9.1)	206 (9.0)
Child-Pugh*		
A	1,889 (76.6)	1,805 (78.7)
B	365 (14.8)	299 (13.0)
C	8 (0.3)	2 (0.1)
BMI, median (IQR)	29.8 (25.7-34.9)	29.8 (25.7-34.8)

ALD, alcohol liver disease; BMI, Body Mass Index; HCV, hepatitis C virus; HBV, hepatitis B virus; IQR, Interquartile range; MASLD, metabolic dysfunction-associated steatotic liver disease. *Thirty-five and 32 cirrhotic participants do not have a Child-Pugh class available in the Overall and Paired Effectiveness populations, respectively. Among Child-Pugh class C participants, 1 participant did not have CP score at time of enrollment, and the other 7 were on the liver transplant list.

Participant Classifications and HCC Characteristics

Characteristics	Overall Effectiveness (N=2,467), n (%)	Paired Effectiveness (N=2,293), n, (%)
Confirmed non-HCC	2,266 (91.9)	2,101 (91.6)
Indeterminate observations	157 (6.4)	151 (6.6)
Confirmed HCC	40 (1.6)	37 (1.6)

HCC Characteristics	Overall Effectiveness (N=40)	Paired Effectiveness (N=37)
Tumor number		
1	34 (85.0)	33 (89.2)
2	5 (12.5)	3 (8.1)
≥3	1 (2.5)	1 (2.7)
Tumor size, cm		
<2	12 (30.0)	11 (29.7)
2-<5	23 (57.5)	22 (59.5)
≥5	4 (10.0)	4 (10.8)
Milan Criteria	28 (70.0)	27 (73.0)

HCC, hepatocellular carcinoma

Summary

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Summary

- Early-detection of HCC may improves patient outcomes[1]
- **Circulating tumor DNA (ctDNA) in the blood provides a minimally invasive approach for early HCC detection[2]**
- Oncoguard test is a blood-based test for HCC detection[3]
- ALTUS prospective clinical validation results will be released tomorrow as a late-breaking abstract and presented on Nov 11th at 11am

HCC: Hepatocellular carcinoma, mt-HBT: multi-target Hepatocellular carcinoma Blood Test

1. Villanueva A. *N Engl J Med*. 2019;380(15):1450-1462. 2.Yang JD, et al. *Semin Liver Dis*. 2019;39(4):452-462. 3. Chalasani NP, et al. *Clin Gastroenterol Hepatol*. 2022:20:173-182.

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ALTUS by the numbers

ALTUS Study Highlights

Sites activated

68

Participants enrolled

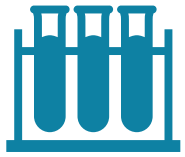
3,089

Forms entered

50,721

Images uploaded

50,704



Tests resulted

2,922

Sites Activated

ALTUS activated 68 sites, ensuring broad access, geographic diversity, and demographic diversity.

Participant Enrollment

ALTUS enrolled 3,089 participants, making it the largest completed study of its kind to date.

Data Completion and Integrity

50,721 forms were entered by sites to collect the necessary data for Enrollment and Visit 1

Images Upload

2,704 images were uploaded for review with 2,572 images passing QC and having reads completed.

Tests Resulted

2,922 tests completed successfully with less than 1% invalid rate.

Variant mRNA in the circulation as a new biomarker analyte

- Aejaz Sayeed, Ph.D. (Blumberg)
- Chen Liu MD PhD (Yale)
- Lewis Roberts MD PhD (Mayo)
- David Kaplan MD (Penn)
- Maarouf Hoteit MD (Penn)
- Dan Zezulinski BS (Blumberg)

Current Blood Analytes

- Protein
- DNA
- Lipid
- MicroRNA

And now…

Current Blood Analytes

- Protein
- DNA
- Lipid
- MicroRNA

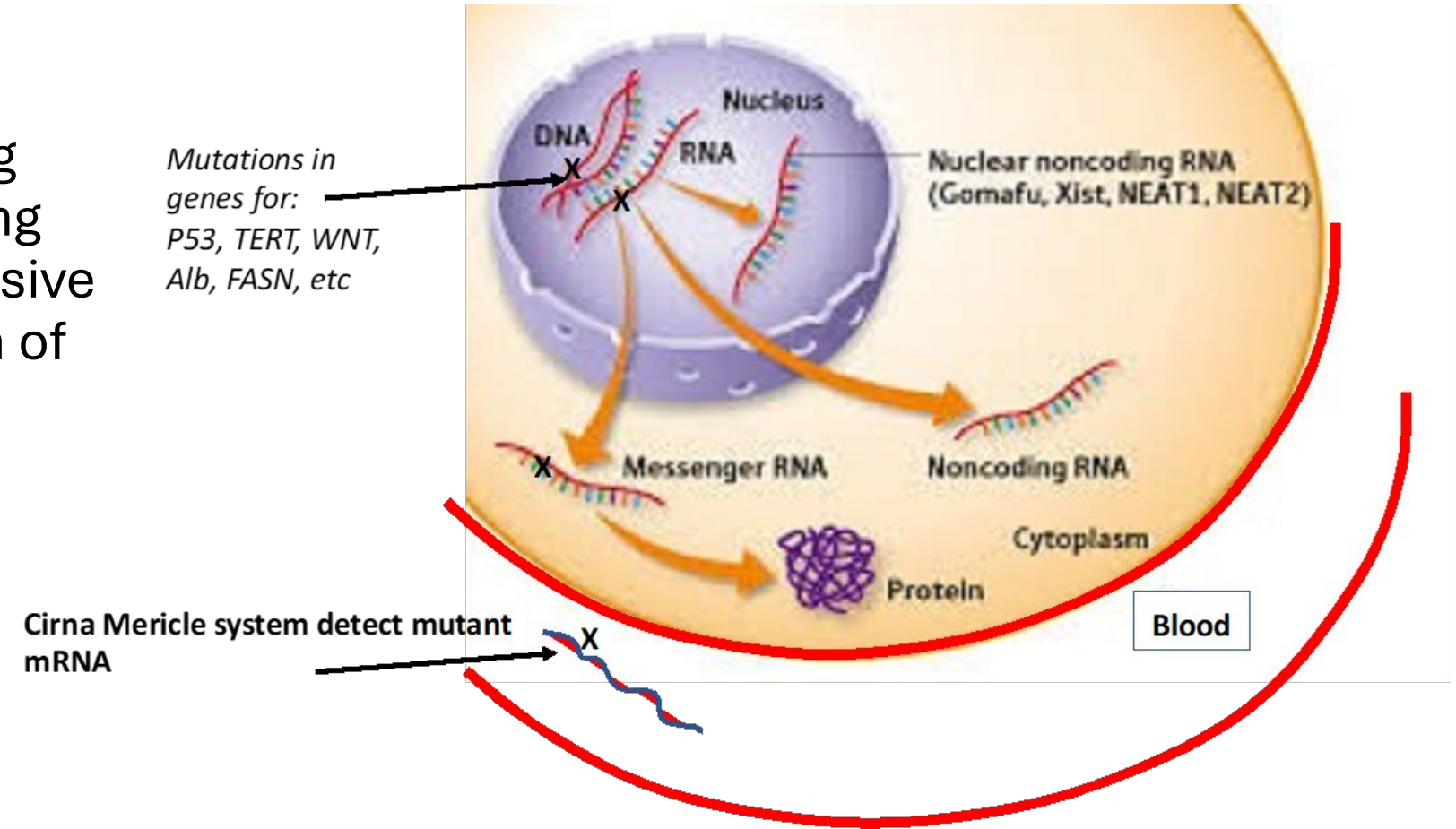
mRNA and LncRNA

Current commercial services testing for blood mRNA and disease

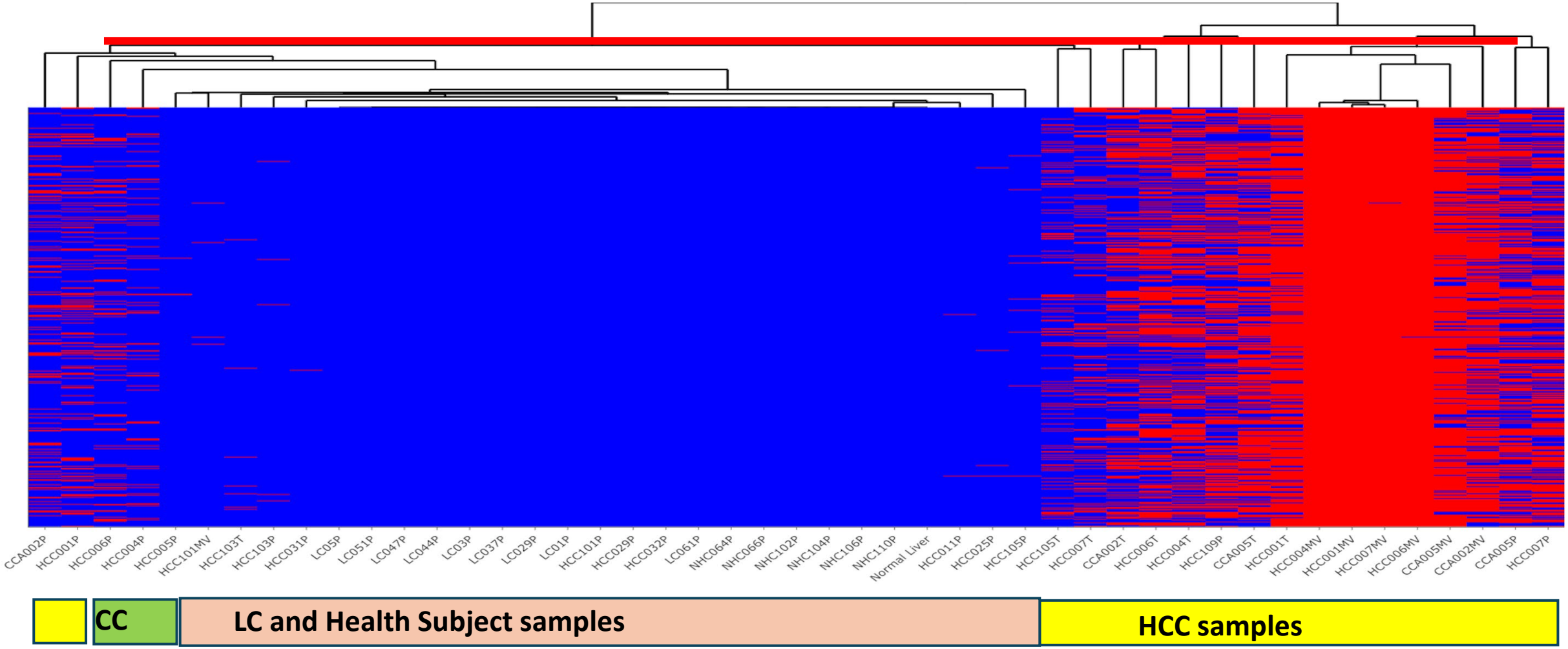
Test/Organ	Company	Genes detected	Indication
Breast			
Syantra DX CE Marked	Syantra Inc	mRNA panel (12 undisclosed markers)	Early detection
BCtect (not FDA)	Digenic ASA	Undisclosed 96 gene + algorithm	Disting benign from malignant
Aristotole (not FDA	Stage Zero	Multipanel, mRNA	Early detection
Prostate			
ProgensaPCA3 (FDA)	Hologic/Gen Probe	PC3mRNA (urine)	Guides biopsy decision
SelectMDx (CLIA)	MDx Health	HoxC6,DLX1mRNA (urine)	Predicts High Grade
ExoDx (EPI) CLIA	Exosome Dioagnostics	PCA3, ERG mRNA (urine)	Predicts need of Biopsy
Lung			
Mayo panel	Mayo Clinic	mRNA fusions	Dx and Rx

But we have shown the blood of people with cancer (HCC) contains variant or mutant mRNA

- Detecting and characterizing these mutations in circulating transcripts offers a non-invasive approach for early detection of patients



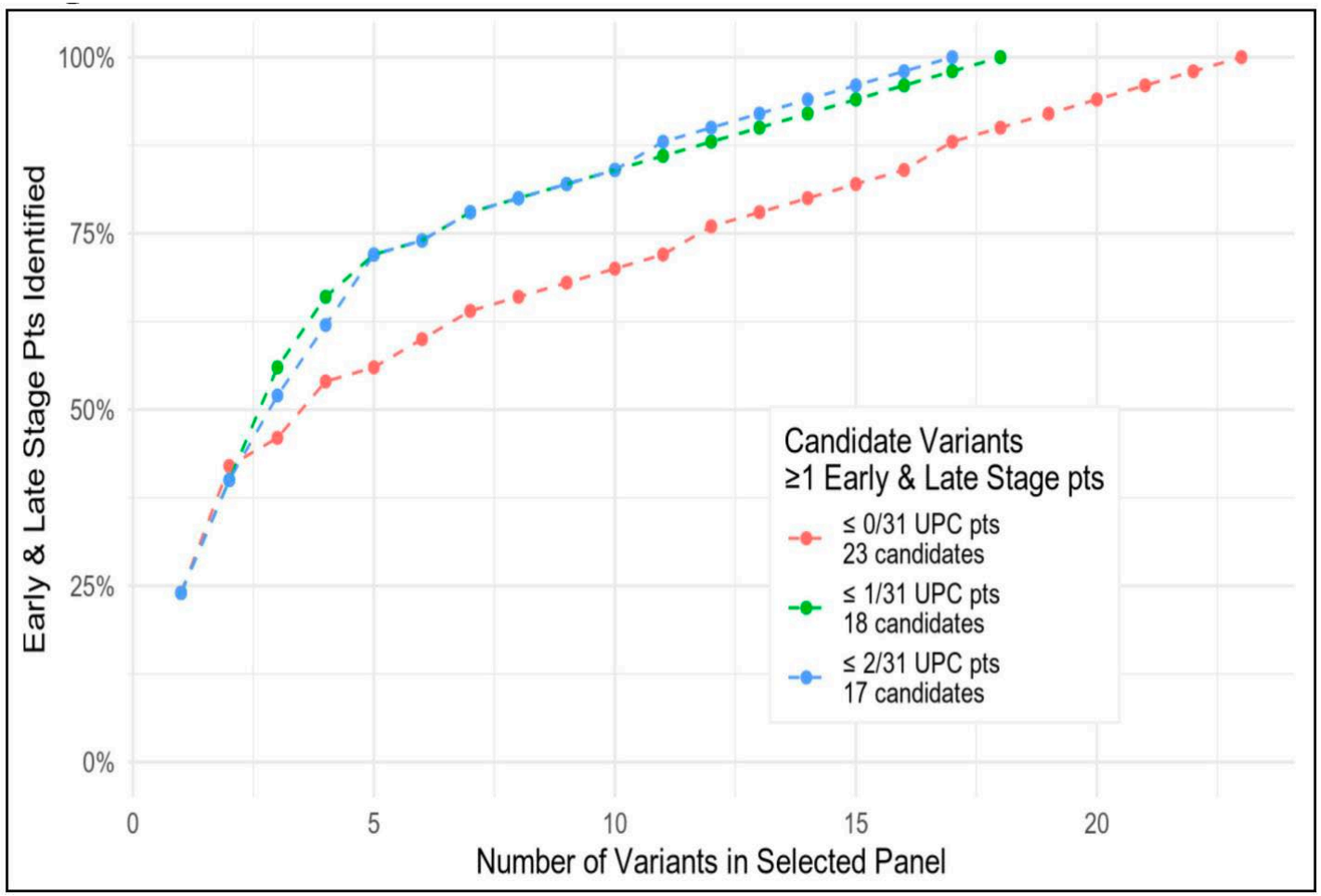
Specific mutant mRNA is in the blood of those with liver cancer, but not healthy subjects



Heat map of 369 high risk mRNA variants (Y axisi) in plasma from HCC ("HCC") (15) but not LC, no HCC ("LC", n=15) and no liver dis ("HS", n=15) plasma and HCC tumors ("T", n=6) See Sayeed 2020. Dendrogram clusters clinical groups by similarity. Each row a different Variant. Since labels for each sample aren't visible, boxes have been inserted to show clinical groups. CC, cholangiocarcinoma

Block T, Zezulinski D, Kaplan DE, Frontiers in oncology. 2022 Dec 13;12:963641.

Initial performance of the HCC panel in distinguishing HCC from LC



Frequent established and previously un-reported variants detected

- **Splice and RNA editing variants**
- **Some associated with drug sensitivity**

Table 4. Annotation of circulating variants exhibiting high diagnostic precision

Gene	variant	dbSNP	effect	Cirrhosis	Early-stage	Late-stage	cancer plasma
ASGR1	chr17:7176997 C>T	SNP	ASGR1:ENST269299.8:	0	0.12	0.36	0.24
MEX3C	chr18:51196776 G>C	SNP	MEX3C:ENST406189.4	0.03226	0.2	0.28	0.24
PDK2	chr17:50094891 GAGGAT>G	INDEL	PDK2:ENST7708.7:c.-2	0.06452	0.16	0.28	0.22
ARCN1	chr11:118583356 G>A	SNP	ARCN1:ENST359415.8:	0	0.28	0.12	0.2
DIAPH1	chr5:141582348 C>G	SNP	DIAPH1:ENST389054.8	0.03226	0.24	0.08	0.16
GDI1	chrX:154442587 T>A	SNP	GDI1:ENST447750.7:c.	0.06452	0.12	0.2	0.16
VAT1	chr17:43015806 C>T	SNP	VAT1:ENST355653.8:c.	0.03226	0.12	0.16	0.14
ARHGAP45	chr19:1080497 C>A	SNP	ARHGAP45:ENST31309	0.03226	0.24	0.04	0.14
PRPF8	chr17:1651476 A>G	SNP	PRPF8:ENST304992.11	0.06452	0.16	0.12	0.14
PTTG1IP	chr21:44856223 C>T	SNP	PTTG1IP:ENST330938.	0.06452	0.12	0.16	0.14
TUBA1C	chr12:49269877 C>T	SNP	TUBA1C:ENST301072.1	0	0.12	0.12	0.12
BIRC2	chr11:102350873 T>A	SNP	BIRC2:ENST227758.7:c	0	0.12	0.08	0.1
TADA3	chr3:9789723 C>T	SNP	TADA3:ENST301964.7:c	0	0.08	0.12	0.1
UBA1	chrX:47199344 G>A	SNP	UBA1:ENST335972.11:c	0	0.08	0.12	0.1
HNRNPL	chr19:38840293 C>T	SNP	HNRNPL:ENST221419.	0.03226	0.12	0.08	0.1
ZNF117	chr7:64978289 G>A	SNP	ZNF117:ENST282869.1	0.06452	0.04	0.16	0.1
EPB41	chr1:29053204 TA>T	INDEL	EPB41:ENST373800.7:c	0	0.04	0.12	0.08
EPB41	chr1:29053290 C>A	SNP	EPB41:ENST373800.7:c	0	0.04	0.12	0.08
GPS2	chr17:7313221 G>A	SNP	GPS2:ENST380728.7:c	0	0.16	0	0.08
P2RY11	chr19:10115113 C>T	SNP	P2RY11:ENST321826.5	0	0.12	0.04	0.08
MRPL4	chr19:10252243 G>T	SNP	MRPL4:ENST590669.5	0	0.08	0.08	0.08
MRPL4	chr19:10252358 G>C	SNP	MRPL4:ENST588502.5	0	0	0.16	0.08
PPP1R12C	chr19:55092500 C>T	SNP	PPP1R12C:ENST26343	0	0	0.16	0.08
DIAPH1	chr5:141583561 T>A	SNP	DIAPH1:ENST389054.8	0	0.08	0.08	0.08
SHARPIN	chr8:144098748 A>T	SNP	SHARPIN:ENST398712.	0	0.08	0.08	0.08
MFN2	chr1:12001541 C>T	SNP	MFN2:ENST000002353	0.03226	0.04	0.12	0.08
STAM	chr10:17693290 A>AA	INDEL	STAM:ENST377524.8:c	0.03226	0.12	0.04	0.08
ARCN1	chr11:118583892 C>A	SNP	ARCN1:ENST359415.8:	0.03226	0.04	0.12	0.08
SRM	chr1:11054996 C>A	SNP	SRM:ENST376957.7:c.6	0	0.08	0.04	0.06
F11R	chr1:160999732 C>T	SNP	F11R:ENST368026.11:c	0	0.08	0.04	0.06
MAP4K2	chr11:64800903 A>C	SNP	MAP4K2:ENST294066.	0	0.12	0	0.06
M6PR	chr12:8946388 C>G	SNP	M6PR:ENST544245.1:c	0	0.04	0.08	0.06
BTBD6	chr14:105249697 C>T	SNP	BTBD6:ENST392554.8:	0	0.04	0.08	0.06
IST1	chr16:71921451 A>C	SNP	IST1:ENST378799.11:c	0	0.04	0.08	0.06
GET3	chr19:12747525 C>A	SNP	GET3:ENST000003573	0	0.12	0	0.06
CHMP2A	chr19:58551755 G>A	SNP	CHMP2A:ENST600118.	0	0.04	0.08	0.06

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The potential of circulating variant mRNA as a biomarker

Compared to DNA:

- ?more sensitive than DNA (mRNA is amplified in number)
- ?detects biomarkers not detectable with DNA (i.e. splice and edited variants)
- Easier to detect than many protein variants

Useful for:

- Disease detection
- Prognosis
- Management and Precision Medicine
- Neoantigens