Oncoguard Liver: Development

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

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

+

Alpha-fetoprotein (AFP)

+

Patient Sex

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.

Oncoguard Liver: Workflow

1 Blood Collection

3 -LBgard® tubes

Methylated DNA Marker analysis

+

SST® tube

AFP analysis

2 Centrifugation

Plasma for Methylated DNA Marker Analysis

Serum for AFP Analysis

3 Methylated DNA Marker Analysis (*HOXA1*, *TSPYL5*, *B3GALT6*)

- cfDNA Extraction
- Bisulfite Conversion
- Methylated DNA Marker Detection

3 AFP Analysis

3 Patient Sex

4 Algorithm
Composite Score

Qualitative Result

\+ -

or Invalid

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

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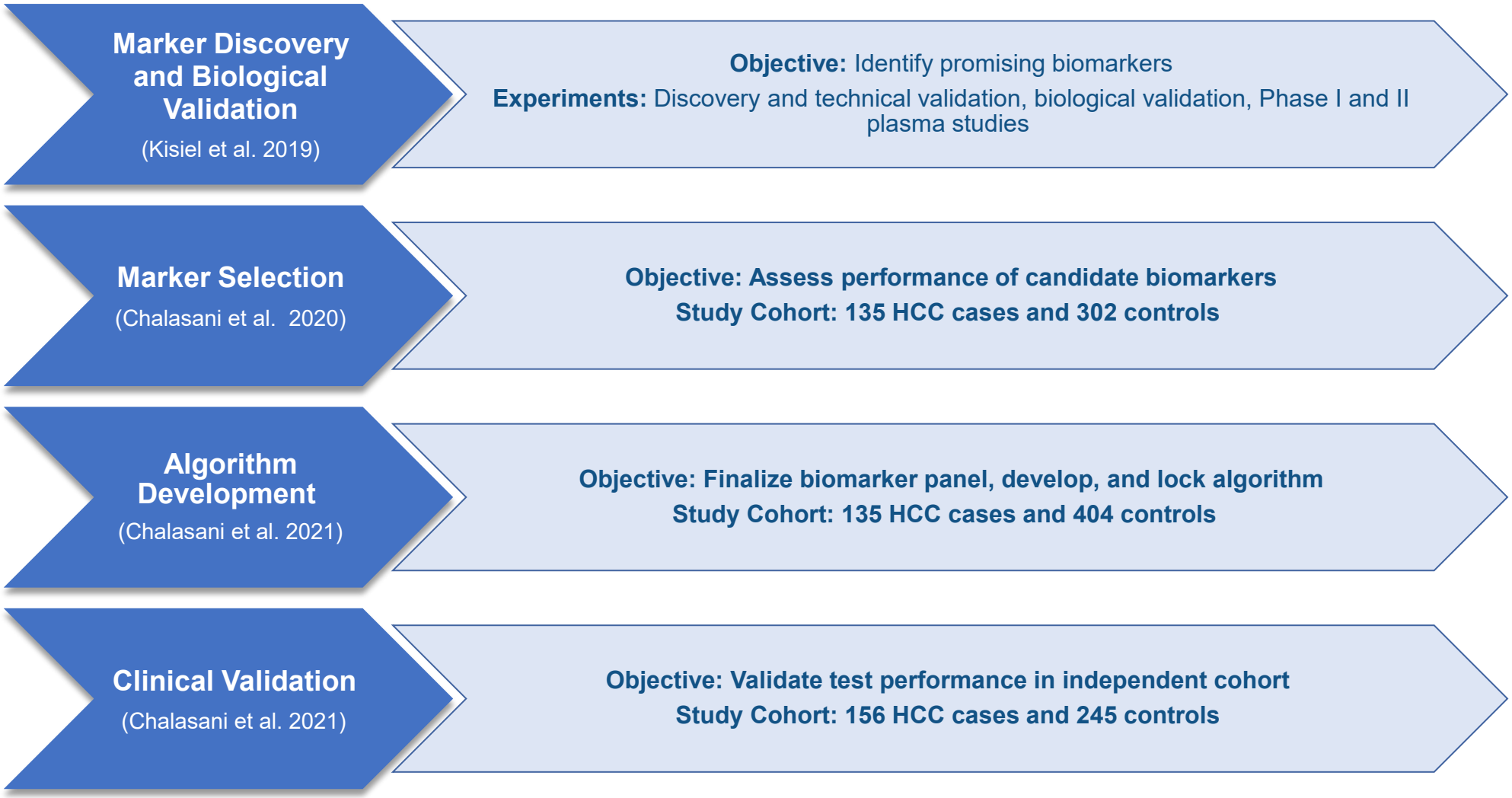
Clinical Development Program



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.

Marker Selection, Algorithm Development and Validation

Design: Prospective, international, multicenter, case-control study of participants ≥18 years of age with untreated clinically diagnosed HCC or negative for HCC undergoing surveillance. Cases included participants with untreated clinically diagnosed HCC and controls were participants who were negative for HCC after disease surveillance.

Sample Collection (NCT03628651)[1]

Marker Selection[2]	Algorithm Development[3]	Clinical Validation[3]	50 additional biopsy-confirmed HCC cases[3]
135 HCC cases	136 HCC cases	159 HCC cases	
302 Controls	404 Controls	250 Controls	
		3 invalid sample results*	
		5 invalid sample results†	
		156 HCC Cases	
		245 Controls	

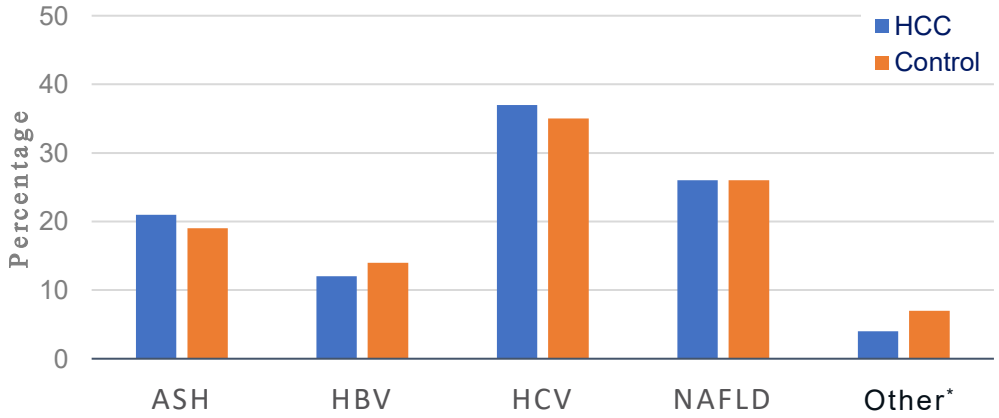
HCC: Hepatocellular carcinoma
*3 invalid sample results for HCC cases (3 related to a run validity failure and insufficient plasma for retesting)
†5 invalid sample results for controls (4 related to a run validity failure and insufficient plasma for retesting, 1 unable to process due to a clot in plasma sample)

1. ClinicalTrials.gov. Blood Sample Collection to Evaluate Biomarkers for Hepatocellular Carcinoma. Updated August 6, 2020. Accessed December 17, 2021. http://clinicaltrials.gov/ct2/show/NCT03628651.
2. Chalasani NP, et al. *Clin Gastroenterol Hepatol*. 2021;19:2597-2605. 3. Chalasani NP, et al. *Clin Gastroenterol Hepatol*. 2022;20:173-182.

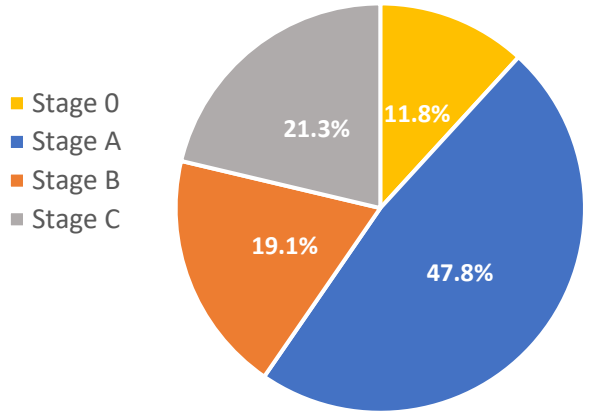
Algorithm Development and Validation - Demographic and tumor characteristics

Algorithm Development

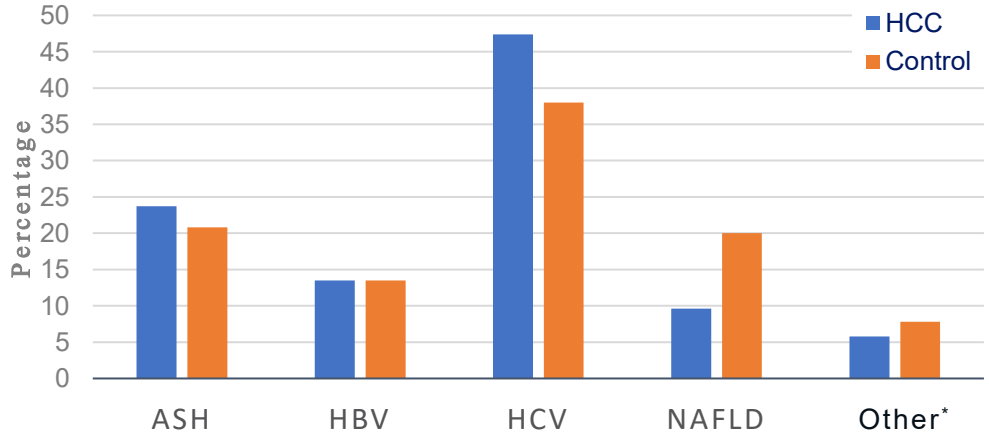
Distribution of Liver Disease Etiologies

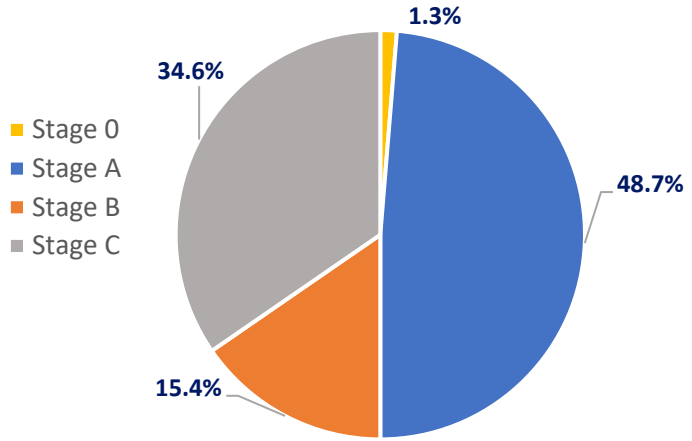


Distribution of HCC cases by BCLC stages



Clinical Validation





ASH: alcoholic steatohepatitis, HBV: hepatitis B virus, HCC: Hepatocellular carcinoma, HCV: hepatitis C virus, NAFLD: Nonalcoholic fatty liver disease, BCLC: Barcelona Clinic Liver Cancer.
*Other: includes autoimmune hepatitis, genetic hemochromatosis, stage 4 primary biliary cholangitis, primary sclerosing cholangitis, and cryptogenic cirrhosis.
Chalasani NP, et al. *Clin Gastroenterol Hepatol*. 2022:20:173-182.

Algorithm Development - Test Performance

	Sensitivity, % (95% CI)			Overall Sensitivity, % (95% CI) (n=156)	Specificity, % (95% CI) (n=245)
	BCLC Stage 0 + A (n=78)	BCLC Stage B + C (n=78)	Milan Criteria (n=68)		
mt-HBT	72 (61, 80)	96 (88, 99)	68 (57, 78)	82 (74, 87)	88 (84, 91)
AFP (≥20 ng/mL)	31 (22, 42)	69 (56, 80)	28 (19, 39)	46 (38, 55)	98 (95, 99)
GALAD (≥-0.63)	67 (56, 76)	91 (80, 96)	64 (52, 74)	76 (69, 83)	86 (82, 89)
GALAD (≥-0.33*)	57 (46, 67)	89 (78, 95)	53 (41, 64)	70 (62, 77)	88 (84, 91)

AFP: Alpha-fetoprotein, BCLC: Barcelona Clinic Liver Cancer, CI: confidence interval, GALAD: Gender, Age, AFP-L3, AFP, and DCP model, mt-HBT: multi-target Hepatocellular carcinoma Blood Test
*Cutoff value corresponds to matching mt-HBT specificity of 88%

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Clinical Validation - Test Performance

	Sensitivity, % (95% CI)			Overall Sensitivity, % (95% CI) (n=156)	Specificity, % (95% CI) (n=245)
	BCLC Stage 0 + A (n=78)	BCLC Stage B + C (n=78)	Milan Criteria (n=68)		
mt-HBT	82 (72, 89)	94 (86, 97)	81 (70, 88)	88 (82, 92)	87 (82, 91)
AFP (≥20 ng/mL)	40 (30, 51)	77 (66, 85)	41 (30, 53)	58 (51, 66)	100 (98, 100)
GALAD (≥-0.63)	71 (60, 79)	91 (83, 96)	71 (60, 80)	81 (74, 86)	93 (90, 96)
GALAD (≥-1.18*)	78 (68, 86)	92 (84, 96)	78 (67, 86)	85 (79, 90)	87 (82, 91)

AFP: Alpha-fetoprotein, BCLC: Barcelona Clinic Liver Cancer, CI: confidence interval, GALAD: Gender, Age, AFP-L3, AFP, and DCP model, mt-HBT: multi-target Hepatocellular carcinoma Blood Test
*Cutoff value corresponds to matching to mt-HBT specificity of 87%

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Clinical Validation - Test Performance Select Subgroups

Subgroup (n)*	Sensitivity, % (95% CI)			Overall Sensitivity, % (95% CI)	Specificity, % (95% CI)
	BCLC Stage 0 + A	BCLC Stage B + C	Milan Criteria		
Sex					
Male (274)	83 (72, 90)	94 (85, 98)	82 (70, 90)	89 (82, 93)	84 (77, 89)
Female (127)	77 (50, 92)	92 (67, 99)	75 (47, 91)	85 (67, 94)	91 (84, 95)
Viral Status†					
Viral (222)	84 (71, 92)	96 (87, 99)	85 (70, 93)	91 (83, 95)	90 (83, 94)
Non-viral (164)	80 (63, 91)	92 (75, 98)	77 (58, 89)	86 (74, 92)	84 (76, 90)
BMI‡					
<30 (229)	85 (72, 93)	96 (86, 99)	86 (73, 94)	90 (83, 95)	87 (80, 91)
≥30 (160)	77 (59, 88)	92 (76, 98)	71 (51, 85)	84 (72, 91)	88 (80, 93)
Child-Pugh class§					
Class A (273)	81 (68, 89)	93 (82, 97)	80 (66, 89)	87 (79, 92)	88 (82, 92)
Class B (96)	81 (60, 92)	96 (78, 99)	79 (57, 92)	88 (76, 95)	83 (71, 91)
Cirrhosis Status					
Cirrhosis (377)	83 (73, 90)	93 (85, 97)	81 (70, 88)	88 (82, 92)	87 (82, 91)

BCLC: Barcelona Clinic Liver Cancer, BMI: Body Mass Index, CI: Confidence Interval, HCC: Hepatocellular carcinoma

*Total number of participants within each subgroup (both HCC and control subjects)
†Viral status was unknown for 6 HCC (1 BCLC stage 0, 2 BCLC stage A, 1 BCLC stage B, and 1 BCLC stage C) and 9 control subjects
‡BMI data were missing for 6 HCC (1 BCLC stage A, 1 BCLC stage B, and 4 BCLC stage C) and 6 control subjects
§Child-Pugh data were missing for 8 HCC (1 BCLC stage 0, 4 BCLC stage A, and 3 BCLC stage C) and 24 control subjects

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Oncoguard™ Liver test (mt-HBT) – Intended Use

- The Oncoguard™ Liver test is a blood-based test for the qualitative detection of biomarkers associated with hepatocellular carcinoma in serum and plasma derived from whole blood.[1]
- The test is intended as an aid in the detection of hepatocellular carcinoma for adults with liver cirrhosis and/or chronic hepatitis B (HBV) who are at risk for hepatocellular carcinoma.[1]
- Guidelines recommend biannual testing in patients at risk for hepatocellular carcinoma.[2,3]
- The Oncoguard™ Liver test was validated in patients at risk for hepatocellular carcinoma, including those with cirrhosis and/or chronic HBV.[1]

HCV: hepatitis C virus, mt-HBT: multi-target-Hepatocellular carcinoma Blood Test, NAFLD: Nonalcoholic fatty liver disease, NASH: Non-alcoholic steatohepatitis

1. Exact Sciences Corporation. Oncoguard Liver Provider FAQs. Revised August 2021. Accessed November 11, 2021. https://www.oncoguardliver.com/providers/faq 2. Heimbach JK, Kulik LM, Finn RS, et al. *Hepatol.* 2018;67(1):358-380.
3. Marrero JA, Kulik LM, Sirlin CB, et al. *Hepatol.* 2018;68(2):723-750.

Summary

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

Additional Evidence
(ALTUS Ongoing)

2019 — 2021

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.

Summary

- Early-detection of HCC may improve patient outcomes[1]
- **Circulating tumor DNA (ctDNA) in the blood provides a minimally invasive approach for early HCC detection[2]**
- The mt-HBT test is a blood-based test for HCC detection[3]
- Clinical validation results showed:[3]
  - Early-stage sensitivity of 82%
  - Overall sensitivity of 88%
  - Specificity of 87%

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.