Early-Stage Detection of Hepatocellular Carcinoma:

The Oncoguard™ Liver Solution

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oncoguard™ liver

EXACT SCIENCES

Executive Summary

Liver cancer has the fastest growing incidence rate of any cancer in the United States and is a leading cause of cancer-related deaths worldwide.[1,2] Up to 85% of all liver cancers are attributed to hepatocellular carcinoma (HCC), which has a five-year survival rate of approximately 20%.[2,3] Given the burden of disease, guidelines in the U.S. currently recommend routine surveillance of individuals at risk for HCC through biannual ultrasound imaging, with or without alpha-fetoprotein (AFP) testing.[4,5] Patient adherence to surveillance, however, is poor, and sensitivity of the recommended methods is suboptimal.[6,7] On average, studies suggest more than 60% of patients are not diagnosed with HCC until the later stages of disease, when treatment options are limited and curative outcomes unlikely.[8,9]

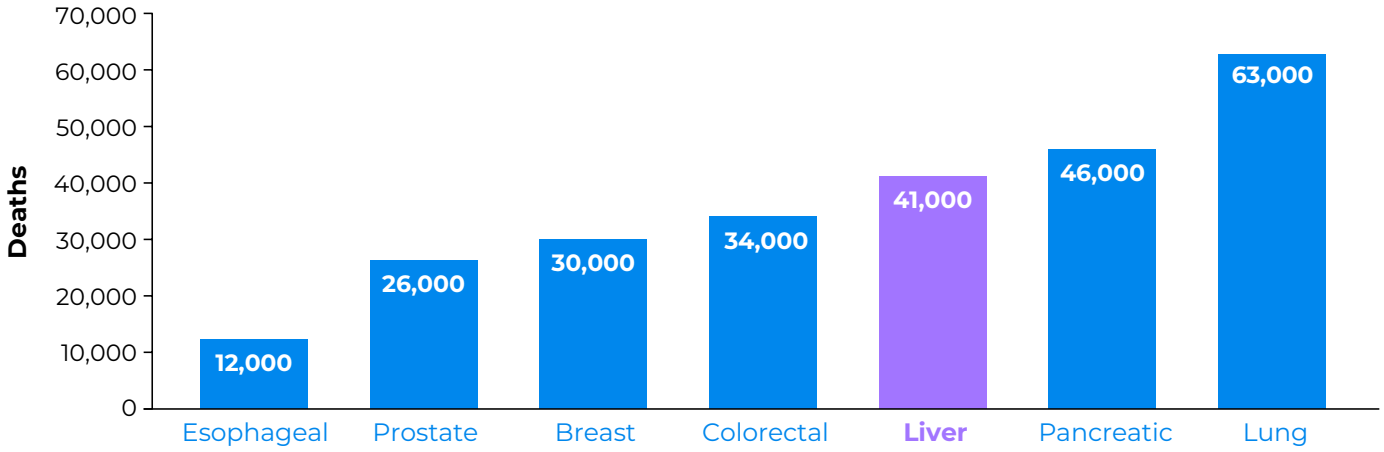
New surveillance strategies that enable the early detection of HCC are key to improving patient outcomes. With this goal in mind, Exact Sciences developed the Oncoguard™ Liver solution, consisting of a blood-based assay for HCC detection and a specially designed patient engagement program. The Oncoguard™ Liver test measures the DNA methylation markers *HOXA1*, *TSPYL5*, and *B3GALT6* and the protein biomarker AFP and incorporates patient sex to classify samples as positive or negative for HCC. Validated using samples collected in a multicenter, case-control study, the test demonstrated enhanced sensitivity for early-stage HCC detection relative to currently available tools such as AFP and GALAD and showed high performance regardless of viral disease status or patient obesity. The test is complemented by a flexible patient engagement program that empowers patients to adhere to surveillance plans with easy-to-understand educational materials and periodic testing reminders. Together, the Oncoguard™ Liver solution provides a comprehensive approach to surveillance and constitutes a breakthrough on behalf of patients with HCC.

Introduction to Liver Cancer: Epidemiology and Burden of Disease

Liver cancer is a leading cause of death worldwide and a fast-growing burden in the United States (U.S.).[1,2] With >40,000 cases and >30,000 deaths reported each year, rates of liver cancer in the U.S. have more than tripled since 1980 and are projected to rise more than 2% with each additional year.[3,10,11] If such increases are sustained, liver cancer will surpass breast and colorectal cancers to become the third-leading cause of cancer deaths in the U.S. by the year 2040 (**Figure 1**).[10]

Rates of liver cancer are on the rise in the U.S. Long-term outcomes are poor, with an overall five-year survival rate of approximately **20%**.[3]

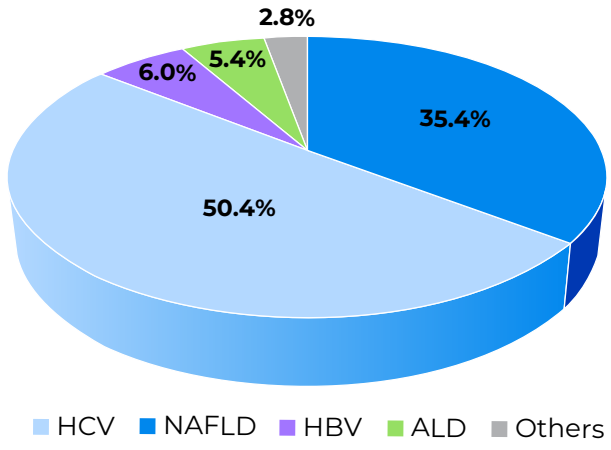
Figure 1. Projected Cancer-Related Deaths in 2040 by Cancer Type[10]



Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma are the two main types of primary liver cancer.[2] Originating in hepatocytes rather than bile duct cells, HCC is the more common neoplasm and comprises up to 85% of total cases.[2,12] Risk factors for HCC have been well established and are known to include hepatitis B virus (HBV), cirrhosis, and genetic conditions that can increase the likelihood of cirrhosis such as hemochromatosis, alpha-1 antitrypsin deficiency, and Wilson's disease.[4,13,14] Cirrhosis typically arises through chronic liver injury and is found to be pre-existing in more than 80% of individuals diagnosed with HCC.[4,14] As a result, any major causes of cirrhosis should also be seen as indirect risk factors for HCC, including HBV, hepatitis C virus (HCV), alcoholic liver disease (ALD), and nonalcoholic fatty liver disease (NAFLD).[4,15] Other factors associated with cirrhosis and HCC include diabetes, smoking, male sex, and older age.[16,17]

While the overall incidence of HCC has been steadily rising, the distribution of underlying etiologies is both geographically heterogeneous and changing over time.[13,18–20] HBV has long been the dominant etiology worldwide, with particularly high prevalence in regions of sub-Saharan Africa and Eastern Asia.[13,19] In recent decades, however, these rates have shown progressive decline in populations with effective vaccine programs and/or new anti-viral therapies.[13,19–22] In the U.S., rates of NAFLD and HCV have been increasing in parallel due to growing obesity and opioid epidemics, respectively.[18,19,23] According to a population-based study spanning from 2007 to 2017, NAFLD and HCV are currently the two major drivers of liver disease mortality in the U.S. (**Figure 2**).[23]

Figure 2. Distribution of Liver Disease Etiologies Leading to Mortality[a]



[a]Based on multiple-cause mortality data from the U.S. National Center for Health Statistics.[23]

ALD, alcoholic liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus; NAFLD, nonalcoholic fatty liver disease.

Early Detection of HCC: Unmet Need and Ongoing Challenge

With the early stages of liver disease characterized by minimal and/or non-specific symptoms, HCC can pose a true diagnostic challenge for physicians.[8,13] In practice, more than 60% of patients with HCC are not diagnosed by a provider until they have reached what are considered the later stages of disease.[8,9] At this point, curative treatments such as liver transplantation and surgical resection are no longer viable options.[13]

As a result, a patient's prognosis is largely dependent on the tumor stage at presentation, as well as a patient's performance status and level of underlying liver function at that time (**Figure 3**).[24] According to data collected by the National Cancer Institute, the five-year relative survival rate of patients with liver cancer is only 2.5% for those diagnosed with distant disease, compared with 12.0% for regional disease and 34.2% for localized disease.[25] In the best-case scenario, survival rates climb even higher: patients who are diagnosed with early-stage disease and eligible for a liver transplant can achieve five-year survival rates over 60% and as high as 78%.[24,26] Calls for improved early detection of HCC have been made in recent years given this difference in outcomes.[27]

Clinical practice guidelines for U.S. physicians currently recommend routine surveillance of patients at risk for HCC based on ultrasound imaging.[4,5] Issued by the American Association for the Study of Liver Diseases (AASLD), the most recent guidelines specify biannual ultrasound exams with or without alpha-fetoprotein (AFP) testing.[4,5] The AASLD also specifies a target population for surveillance that includes adults with cirrhosis and select cases of HBV without cirrhosis. In both populations, positive screens should be followed by diagnostic imaging of the abdomen using multiphasic computed tomography or magnetic resonance imaging.[4,5]

When used, these methods of surveillance can nearly double the three-year survival rate of patients with HCC.[7] Unfortunately, studies indicate that many providers are not up-to-date with the issued guidance, and less than 30% of eligible patients receive the recommended surveillance.[6,28] Several test-specific limitations have also been reported. Ultrasound, for example, is operator-dependent and performs poorly in obese populations and individuals with non-alcoholic steatohepatitis (NASH) or cirrhotic heterogeneous livers.[24,27,29,30] **As many as one in five ultrasound exams are thought to be of inadequate quality for analysis.**[31]

Guidelines intend for AFP testing to accompany, not replace, ultrasound. Nevertheless, reports show that AFP is the sole surveillance tool used in as many as 46% of cases.[32,33] The utility of this approach is controversial, with evidence suggesting AFP is not elevated in all cases of HCC and demonstrates poor sensitivity (32–49%) in patients with early-stage disease.[27,34,35]

GALAD (Gender, Age, Lectin-bound AFP, AFP, Des-carboxy-prothrombin) is a newer model that was designed to improve upon AFP-only testing.[36,37] The GALAD score may be difficult to interpret because of variability associated with patient characteristics and underlying risk factors, and it has not been guideline-recommended for HCC surveillance to date.[38–40]

Routine surveillance and early detection are the keys to better patient outcomes. Patients diagnosed with early-stage HCC can achieve five-year survival rates beyond 70% when undergoing surgical resection or liver transplantation, compared with a median survival of approximately 1 year for patients with advanced HCC.[9,26]

Figure 3. Stages of Hepatocellular Carcinoma

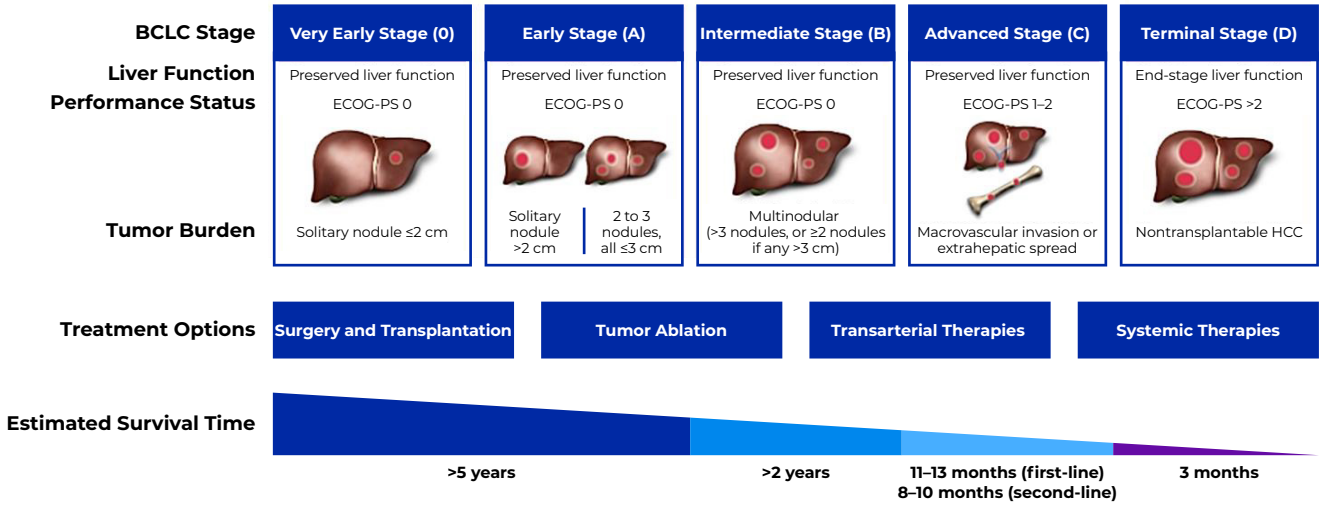


Figure reprinted with permission from Massachusetts Medical Society.[24]
BCLC, Barcelona Clinic Liver Cancer; ECOG-PS, Eastern Cooperative Oncology Group Performance Status.

The Oncoguard™ Liver Solution: A New Approach to HCC Surveillance

Over the last decade, liquid biopsy has emerged as a promising new tool for the detection of multiple cancer types.[41,42] This approach represents a minimally-invasive alternative to surgical biopsy and involves sampling and analyzing an individual's blood, urine, saliva, or other body fluid for the presence of informative biomarkers.[41,43] In oncology, most liquid biopsies target biomarkers that are shed by a tumor and released into the bloodstream, including those from circulating tumor cells, circulating tumor DNA (ctDNA), and extracellular vesicles.[43] In HCC, this can also include methylated DNA markers (MDMs) — a subset of ctDNA showing aberrant methylation of gene promoter regions.[44,45]

Researchers at Exact Sciences recently developed a high-performance assay for HCC surveillance using this technology. Known as the Oncoguard™ Liver test, the innovative new tool is a simple-yet-sophisticated blood test that predicts the likelihood of HCC using a combination of methylated DNA and protein biomarkers. The Oncoguard™ Liver test was created in collaboration with leading experts at the Mayo Clinic and built on a clinical development platform designed to ensure robust results with real-world applicability.

The development process began in the biomarker discovery phase, with the identification of >300 potential MDMs.[46] Candidate regions were selected when there was evidence of differential hypermethylation across case and control samples and underwent biological validation in independent tissue samples. MDMs were further interrogated in phase 1/2 studies using samples of blood plasma rather than frozen tissue,[46] and a subset of 10 high-performing MDMs were ultimately chosen for further evaluation.

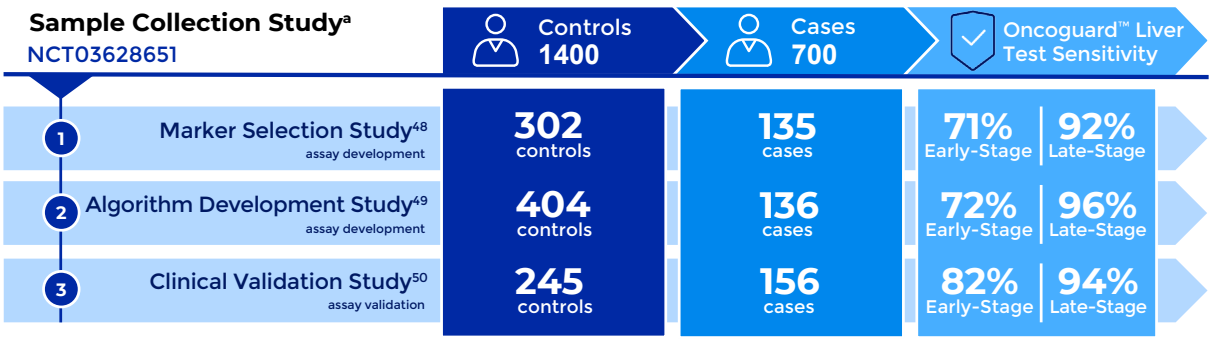
To enable further evaluation of potential markers, a multicenter study was conducted for the purpose of large-scale data collection (ClinicalTrials.gov, NCT03628651).[47] The study enrolled a combined 700 cases and 1400 controls across sites in the U.S., Europe, and Asia, and represents one of the largest case-control studies of HCC in recent history. Specimens collected as part of this effort were then used across multiple phases of product development, including marker selection, algorithm development, and clinical validation studies (**Figure 4**). In all phases, cases were defined as individuals with untreated HCC who had been clinically diagnosed within the past six months and controls as individuals with a definitive lack of HCC who were undergoing surveillance imaging procedures. Unique samples were used for each phase of development and carefully selected to be representative of the distribution of disease etiologies found in the U.S. (ie, HCV, NAFLD, ALD, HBV).

Results were consistent across all phases of development and highlight the test's ability to provide reliable results for a broad group of patients with varied etiologies of disease. During **MARKER SELECTION**, the 10 MDMs identified previously were analyzed along with three protein biomarkers. Copy numbers and protein values were entered into a logistic regression analysis and used to classify samples as either positive or negative for HCC. This initial analysis identified three MDMs (*HOXA1*, *EMX1*, *TSPYL5*), two protein biomarkers (AFP, AFP-L3), and one reference DNA marker (*B3GALT6*) as contributing variables. When combined, the newly developed panel demonstrated 71% early-stage sensitivity and 92% late-stage sensitivity at 90% specificity.[48]

The panel was refined during the subsequent **ALGORITHM DEVELOPMENT** phase and a simpler model with only five predictors (*HOXA1*, *TSPYL5*, *B3GALT6*, AFP, and patient sex) was selected. Results were consistent with the initial findings, showing 72% early-stage sensitivity and 96% later-stage sensitivity at 88% specificity.[49] The optimized panel demonstrated higher sensitivity for early-stage HCC compared with either AFP (31% at a cutoff of 20 ng/mL) or GALAD (67% at a cutoff of –0.63) and similar performance across patients with both viral and non-viral disease status.[49]

The five-marker panel underwent **CLINICAL VALIDATION** in the final phase of development. Blood specimens from 156 cases and 245 controls were analyzed using the model established in the algorithm development study.[50]

Figure 4. The Development Journey of the Oncoguard™ Liver Test



[a]The sample sizes for the marker selection, algorithm development, and clinical validation studies do not sum to the total number of subjects enrolled in the sample collection study (NCT03628651).

Table 1. Comparative Test Performance

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

AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; GALAD, Gender, Age, Lectin-bound AFP, AFP, Des-carboxy-prothrombin.

Similar to earlier results, the panel showed 82% early-stage sensitivity and 94% later-stage sensitivity at 87% specificity (**Table 1**).[50] Additional analyses comparing the Oncoguard™ Liver test to currently available surveillance tools were also conducted. Results indicated that sensitivity of the Oncoguard™ Liver test (82%) was superior to both AFP (40% at a cutoff of 20 ng/mL, p<0.001) and GALAD (71% at a cutoff of –0.63, p=0.03) for patients with early-stage HCC. The test also demonstrated enhanced sensitivity (81%) for patients within the Milan criteria (ie, a single tumor ≤5 cm in size or up to three nodules ≤3 cm in size without macrovascular invasion or extrahepatic spreading) relative to historic ultrasound performance (~47% per a recent meta-analysis).[51] Sensitivity of the Oncoguard™ Liver test was similar regardless of viral disease status or patient obesity (body mass index <30 or ≥30 kg/m^2) (**Table 2**).[50]

82%

The Oncoguard™ Liver test maintains high sensitivity (ie, approximately 82%) at the early stages of disease, when diagnosis is at its most critical. A clinical validation study shows that performance is also high across important subgroups, making the Oncoguard™ Liver test a logical choice for real-world clinical practice.

With rigorous methodology leading to consistent results across all phases of development, physicians can be confident the Oncoguard™ Liver test will translate into real-world practice and provide substantial benefit to their patients at risk for HCC.

Performance, Access, Adherence: A Three-Pronged Approach to HCC Surveillance

A high-performance assay has little value if it cannot be implemented successfully, and real-world data suggest that access and adherence are both significant problems for today's patient.[6,52,53] Indeed, while an estimated three million patients qualify for HCC surveillance in the U.S., a recent meta-analysis suggests surveillance utilization is currently less than 30%.[6] Survey-based studies have reported several barriers to surveillance completion, including scheduling difficulties, transportation problems, and costs associated with testing.[52] Adherence can also depend on a patient's understanding of disease risk and a provider's opportunity to educate the patient given limited time and competing clinical concerns (e.g., heart disease, diabetes).[52,53]

The Oncoguard™ Liver solution is designed to address these issues. The test itself is a simple blood test and requires only a single visit to complete. The blood can be collected in a provider's practice or an associated laboratory, which offers potentially greater convenience

Table 2. Clinical Validation Performance in Select Subgroups

	Subgroup	Sensitivity, % (95% CI)				Specificity % (95% CI)
		Milan Criteria	Early-Stage (BCLC 0 + A)	Later-Stage (BCLC B + C)	Overall	
Viral Status[a]	**Viral**	85 (70–93)	84 (71–92)	96 (87–99)	91 (83–95)	90 (83–94)
	Non-viral	77 (58–89)	80 (63–91)	92 (75–98)	86 (74–92)	84 (76–90)
Obesity[b]	**BMI <30 kg/m^2**	86 (73–94)	85 (72–93)	96 (86–99)	90 (83–95)	87 (80–91)
	BMI ≥30 kg/m^2	71 (51–85)	77 (59–88)	92 (76–98)	84 (72–91)	88 (80–93)

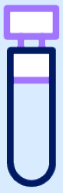
[a]Viral status unknown for 6 patients with HCC and 9 control subjects; [b]BMI data missing for 6 patients with HCC and 6 control subjects.
BCLC, Barcelona Clinic Liver Cancer; BMI, body mass index.

to patients than the imaging procedures that are today's standard of care. In an effort to help empower patients to take control of their own care, the Oncoguard™ Liver solution also includes a complementary patient engagement program. Components of the specially designed program include an informational brochure, mailed outreach materials, and periodic re-screen reminders, all written in patient-friendly language. Providers also receive periodic reports of patients who may qualify for surveillance and be due for testing. Tailored approaches like these (e.g., electronic reminders, mailed outreach) have been shown to increase adherence to surveillance by over 50% in previous studies[6,54,55] and set the Oncoguard™ Liver solution apart from currently available tools.

Summary

Routine surveillance is the key to early detection and curative outcomes in patients with HCC. In the current landscape, however, less than 30% of patients receive the recommended surveillance for HCC and more than 60% are not diagnosed until the later stages of disease.[6,8,9] With this in mind, Exact Sciences developed the Oncoguard™ Liver solution: a comprehensive approach to surveillance that integrates a novel blood-based assay for HCC detection with a tailored patient engagement program for patient adherence. The clinically validated test uses a combination of DNA methylation markers, AFP, and biological sex to identify patients in need of additional diagnostic follow-up and demonstrates enhanced sensitivity for the detection of early-stage disease relative to currently available tools. A unique patient engagement program complements the test's high performance and features patient-friendly educational content and periodic re-screen reminders to help increase surveillance adherence. Together, the Oncoguard™ Liver solution represents a step forward on behalf of patients with liver disease who are at elevated risk of developing HCC.

Current approaches to surveillance are both underutilized and underperforming. The Oncoguard™ Liver solution is a newly developed blood-based liquid biopsy assay that incorporates methylated DNA and protein biomarker analyses, shows enhanced early-stage sensitivity compared with the current guideline-recommended blood test, and comes with a complementary patient engagement program designed to increase surveillance adherence.



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