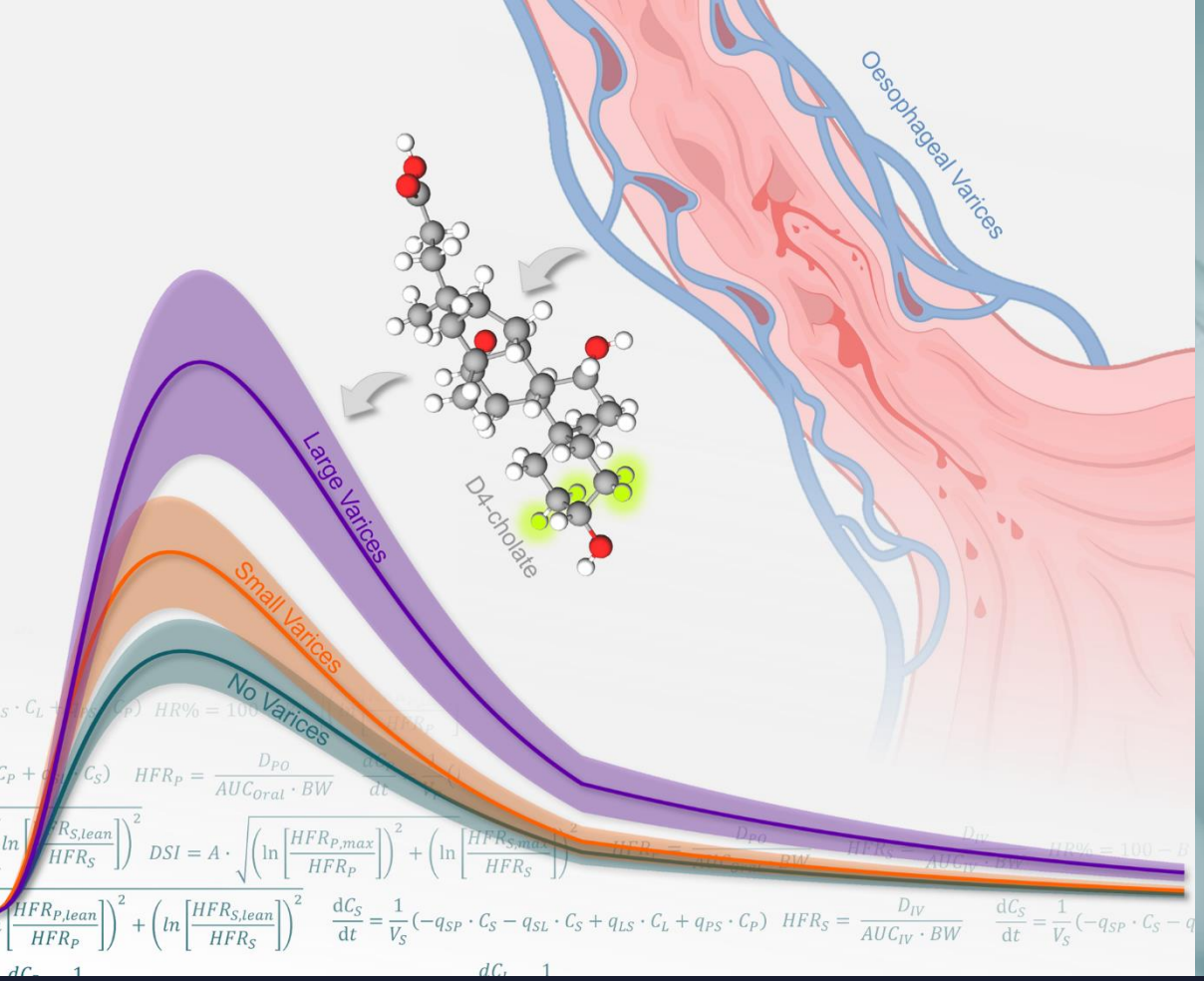


HepQuant DuO™ Defines Risk for Clinical Outcome and Quantifies Changes in Risk

Gregory T. Everson, MD

Liver Forum
September 10, 2025

HEPQuant™
Transforming Liver Health

Disclosures

- Dr. Gregory T. Everson, MD, is an Emeritus Professor of Medicine at the University of Colorado School of Medicine and Chief Executive Officer of HepQuant LLC.
- Dr. Everson is the inventor of the HepQuant technology, founder of HepQuant LLC, and holds intellectual property rights to HepQuant technology. He is a paid employee of HepQuant LLC.

Advantages of HepQuant DuO for the Clinic and Clinical Trials

1. Quantifies liver health by measuring liver function and physiology
   - Hepatocyte function (cholate clearance)
   - Portal-systemic shunting
2. Characterizes functional heterogeneity in the study population
3. Detects in “Real time”
   - Treatment effects – particularly in the early course of therapy
   - Dose-response and time action of administered drug
4. Links treatment risk for clinical outcome
5. Stratifies clinical risk, allowing for efficient study design: can reduce trial duration and sample size

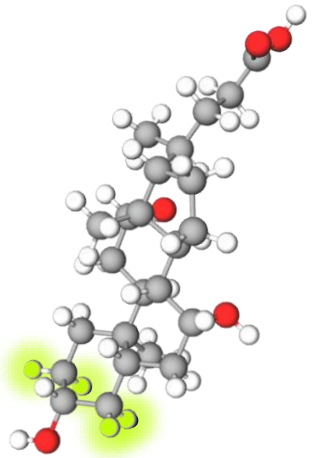
The HepQuant DuO Test

Liver Health Quantified

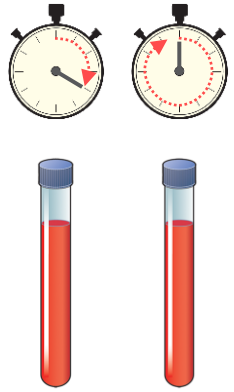
Hepatocyte uptake
Hepatic and Portal blood flow
Portal-systemic shunting

Generating Test Results

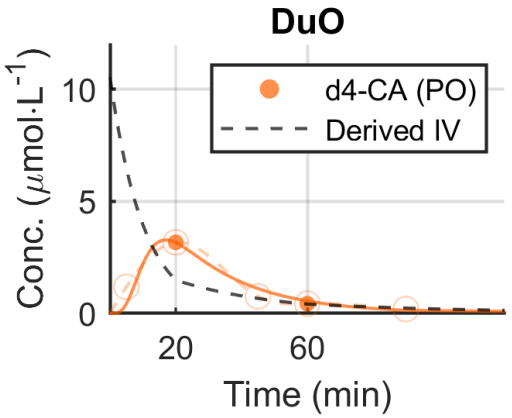
Oral dose of d4-cholate



Two blood draws at 20 & 60 min



Measure cholate clearance by LC-MS/MS



Measures of Liver Health

Disease Severity Index (DSI)

Hepatic Filtration Rates

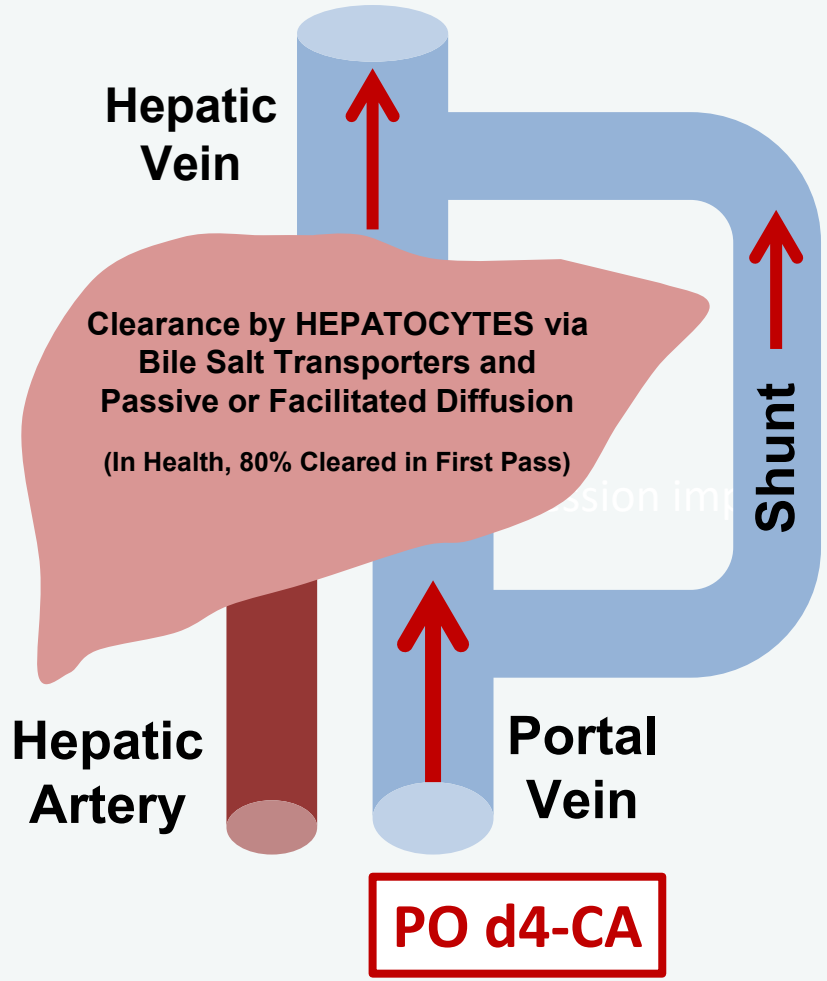
SHUNT%

Hepatic Reserve

RISK ACE

HEPQuant Transforming Liver Health

Impact of Disease Progression on Test Results



Disease Progression impairs hepatocyte function and increases portal-systemic shunting

- **Disease progression increases:**
  - **[d4-CA] in peripheral venous blood**
  - **DSI**
  - **SHUNT%**
- **Disease progression decreases:**
  - **Hepatic Filtration Rate**
  - **Hepatic Reserve (HR%)**

HEPQuant Transforming Liver Health

How Are HepQuant DuO Results Applied in Practice?

HepQuant DuO Test Result

Disease Severity Index (DSI)

» Scaled from 0 to 50
» Healthy lean individuals have DSI < 11.3

→

- Risk of portal hypertension
- Risk of esophageal varices
- Risk of clinical outcomes

Hepatic Reserve (HR)%

» Scaled from 100 to 0
» Healthy Lean individuals have HR > 97.3%

→

- Patient metric – how much healthy liver do I have left?

SHUNT%

» Scaled from 0 to 100
» Healthy lean individuals have SHUNT% < 23.7%

→

- Measures portal-systemic shunting

1. Hassanein T, Keaveny AP, Mantry P, et al. Liver function and portal-systemic shunting quantified by the oral cholate challenge test and risk for large oesophageal varices. Aliment Pharmacol Ther. 2024; 60(2):246-256. 2. Shiffman M, Reddy KR, Leise MD, et al. Cholate Shunt, Oral Cholate Challenge and Endoscopic Lesions of Portal Hypertension: The SHUNT-V Study. Aliment Pharmacol Ther. 2025;61(1):75-87. 3. Kittelson J, McRae MP, Everson GT. Measuring the risk of clinical adverse events (RISK ACE) by quantifying liver function: A patient-centric model. Eur J Int Med 2025;132:160-163.

HEPQuant™ Transforming Liver Health

Identifying the High Risk Patient

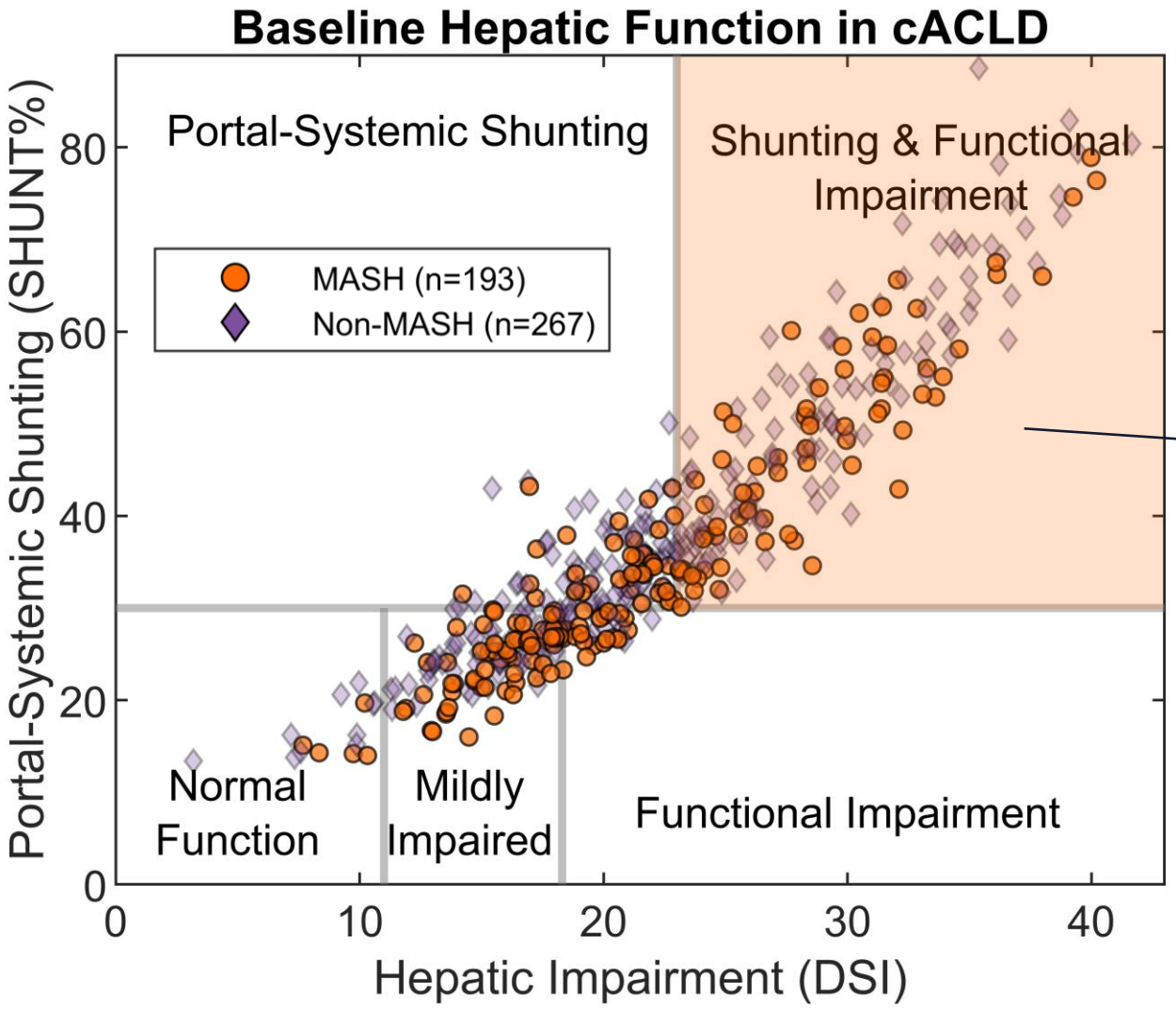
Subjects with Child-Pugh A Cirrhosis have a Broad Range of DSI

Mean DSIs across all studies was similar but SDs relatively large

Trial	Description	n	Baseline DSI (Mean ± SD)
Trial 1 (HCV)	HALT-C	93	22.5 ± 6.1
Trial 2	SHUNT-V		22.6 ± 7.5
MASH		114	21.7 ± 7.0
Non-MASH		124	23.3 ± 7.9
Trial 3 (MASH)	Pharma study	28	22.5 ± 7.4
Trial 4 (MASH)	Pharma study	35	19.3 ± 4.1
Trial 5			19.9 ± 6.7
MASH		8	21.3 ± 6.2
Non-MASH		27	18.8 ± 6.5
Trial 6	Pharma study	23	25.4 ± 5.8
Trial 7 & 8 (MASH)	Pharma study	8	21.2 ± 3.9
All Subjects		460	22.2 ± 6.9
MASH		193	21.5 ± 6.5
Non-MASH		267	22.7 ± 7.2

HepQuant Data on File

DSI >24 and SHUNT% >30% Identify the Most Impaired Subjects

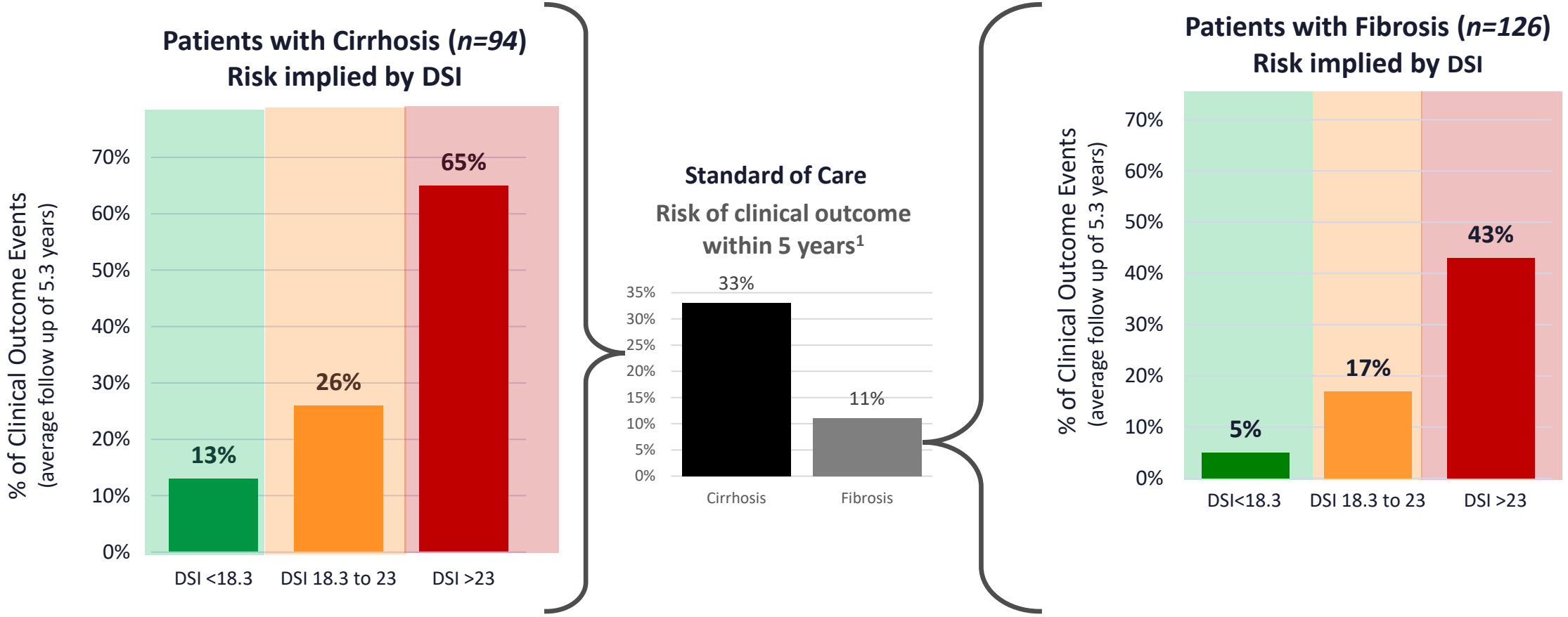


	All	Non-MASH	MASH
DSI >24	35%	38%	31%

HepQuant Data on File

Linking Risk to Clinical Outcome

Quantitative Liver Function Sub Study: Role of Disease Severity Index, Linked to Risk for Clinical Outcome

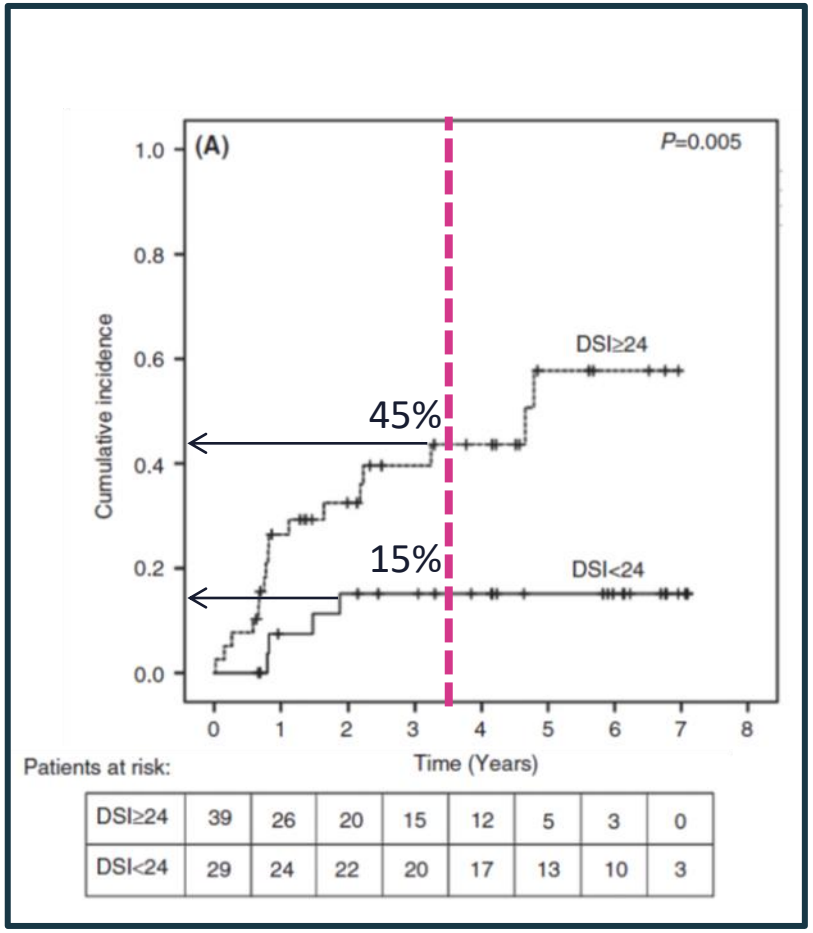


DSI implied risk reflects the percentage of patients in the study that experienced clinical outcomes (ascites, encephalopathy, spontaneous bacterial peritonitis, variceal hemorrhage, or liver related death) within 5.3 years[2]

Dienstag J, et al. Prospective Study of the Rate of Progression in Compensated, Histologically Advanced Chronic Hepatitis C (QLFT cohort). Hepatology 2011;54:396-405. Hassanein T, et. al. Liver Function and Portal-Systemic Shunting Quantified by the Oral Cholate Challenge Test and Risk for Large Esophageal Varices. *Aliment Pharmacol Ther 2024; DOI: 10.1111/apt.18054. Kittelson J, et al. Measuring the risk of clinical adverse events (RISK ACE) by quantifying liver function: A patient-centric model. Eur J Int Med 2025;132:160-163*

DSI 24 as a cutoff for Clinical Outcome in Cirrhosis

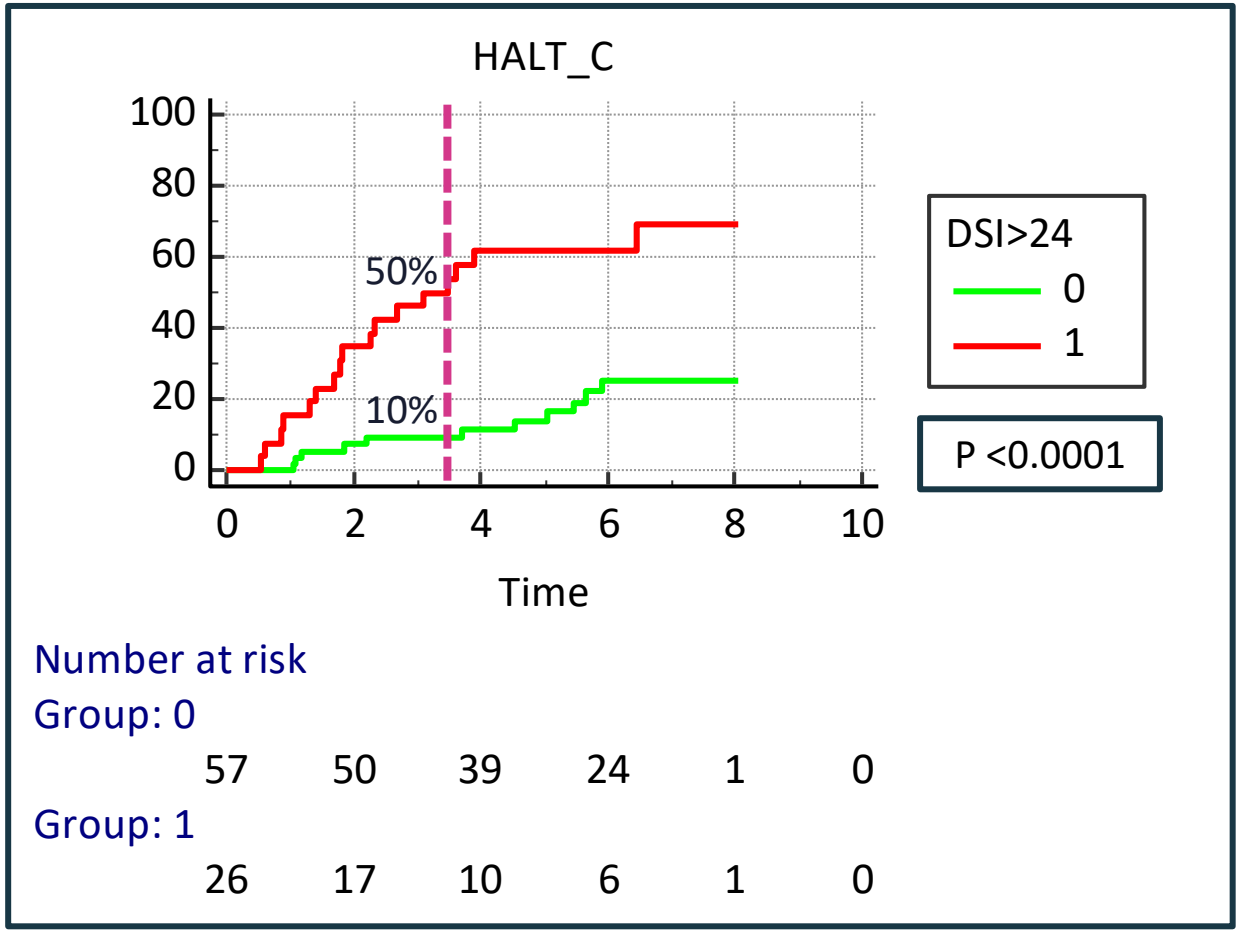
All Etiologies CPA Cirrhosis*



DSI≥24	39	26	20	15	12	5	3	0
DSI<24	29	24	22	20	17	13	10	3

Spectrum of Etiologies of Cirrhosis (20% had DSI>35; 13% had DSI<15)

HCV CPA Cirrhosis



HCV Etiology of Cirrhosis (2% had DSI>35; 8% had DSI<15)

*Predicting clinical decompensation in patients with cirrhosis using the Hepquant-SHUNT test. Fallahzadeh MA, et al. Aliment Pharmacol Ther. 2021;53:928–938.

Estimating Clinical Outcome and Disease Progression using DSI and ΔDSI (RISK ACE)

RISK ACE – DSI Cutoffs for Risk

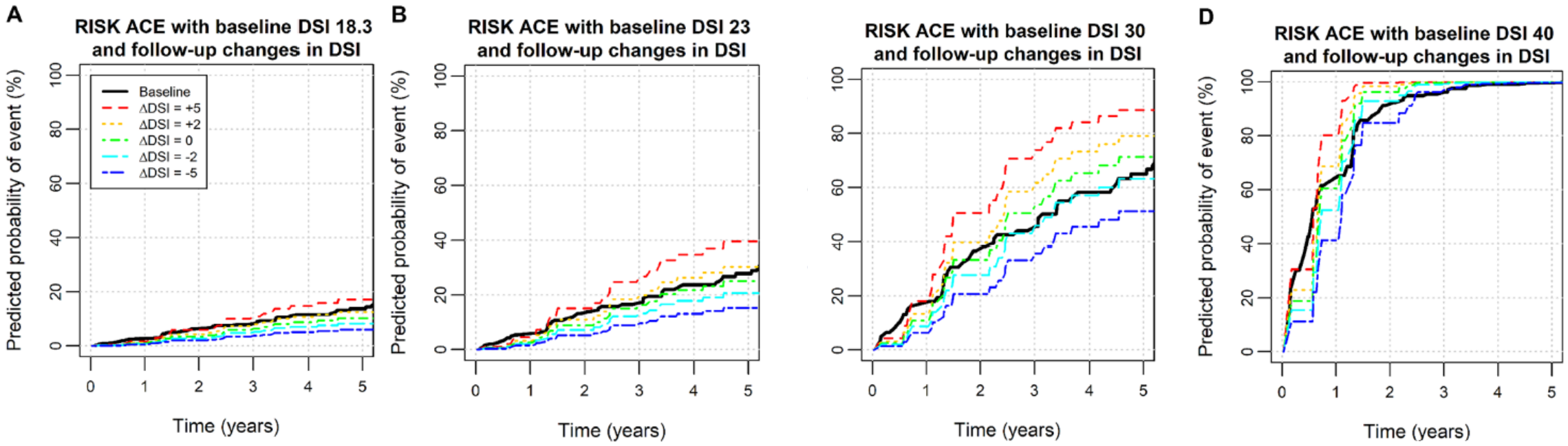
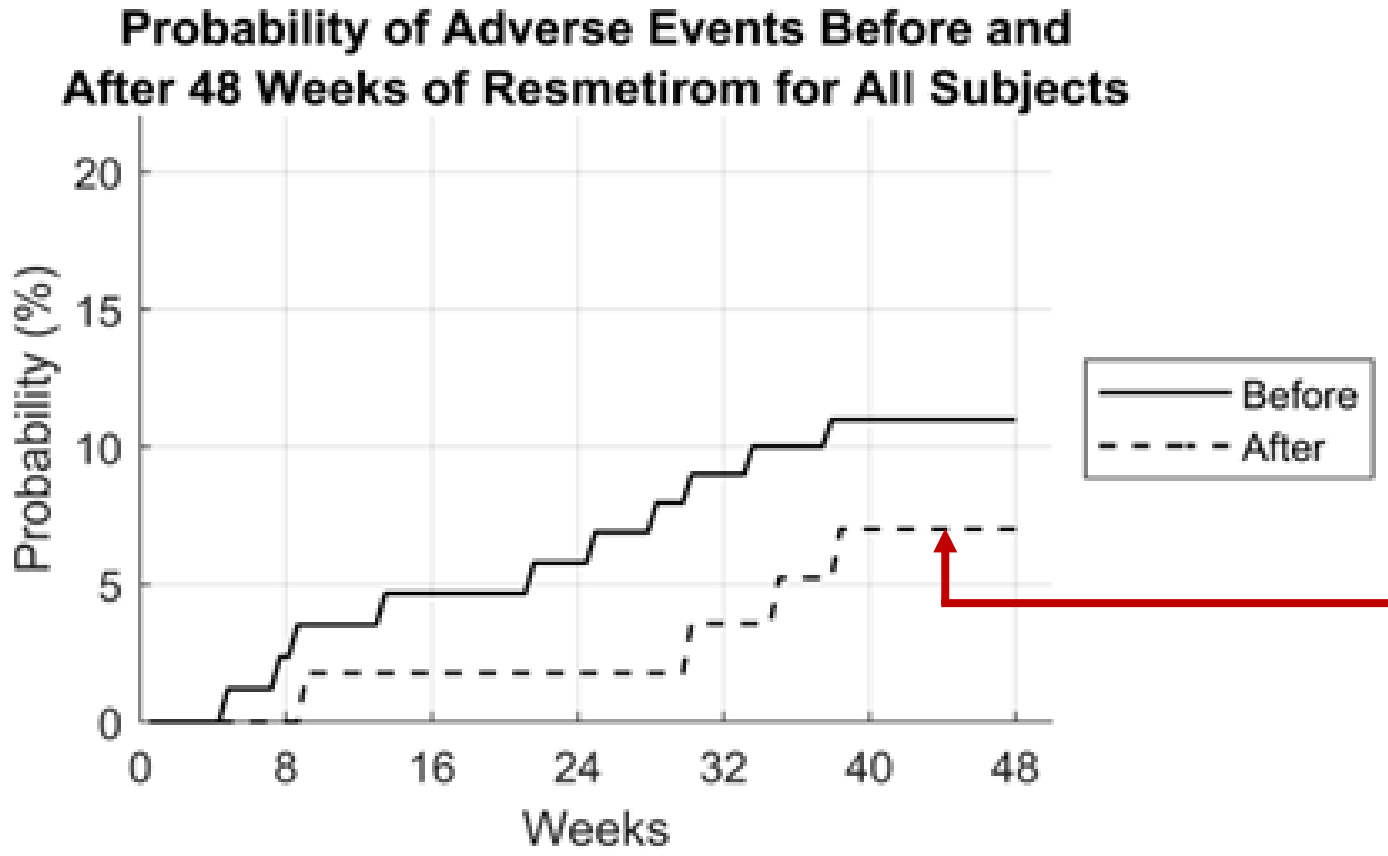


Fig. 1. Evaluation of predicted risk for adverse clinical events (RISK ACE) with various changes in disease severity index (DSI) after approximately two years follow-up. The RISK ACE BASELINE model (solid black line) was evaluated with baseline DSI values of 18.3 (A), 23 (B), 30 (C), and 40 (D), with predicted probabilities expressed in terms of time from the baseline test. The RISK ACE FOLLOW-UP model (colored lines) was evaluated using the corresponding baseline DSI values and with various changes from baseline at follow-up, with predicted probabilities expressed in terms of time from the follow-up test.

Kittelson J, et al. Measuring the risk of clinical adverse events (RISK ACE) by quantifying liver function: A patient-centric model. Eur J Int Med 2025;132:160-163.

HepQuant's RISK ACE Defines Treatment Effect
Estimated Risk Reduction in Clinical Outcome*



Estimated outcome rate for resmetirom treatment **7%**
(vs. 11% untreated)

- **RISK ACE decreased after week 48 of treatment in 19 of 23 subjects, with a significant reduction in absolute risk of −4.0%, p=0.041**

Alkhouri N, McRae MP, Taub R, Hill B, Imperial JC, Kittelson J, Moussa SE, Everson GT. The cholate Challenge test characterizes disease severity in MASH-related Child Pugh A cirrhosis and potential clinical benefit of resmetirom. Gastro Hep Advances. 2025. Available online.

Estimated Outcome from RISK ACE matched Observed Outcome

Safety Summary after 2-year Open-Label Treatment with Resmetirom

Summary AEs (2 years of treatment)	Resmetirom (n=122)
Any TEAE	113 (93%)
Any SAE	27 (22.1%)
TEAE leading to Trial Discontinuation	3 (2.5%)
Death[1]	2 (1.6%)
Common AEs[2]	**Resmetirom (n=122)**
AE occurring in >15% of patients	
Diarrhea	46 (38%)
Covid-19	38 (31%)
Nausea	38 (31%)
Urinary Tract Infection	33 (27%)
Headache	21 (17%)
Arthralgia	19 (16%)
Fatigue	19 (16%)
Pruritus	20 (16%)
Vomiting	18 (15%)

Data are n (%)

- Safety data were consistent with previous studies
- Resmetirom was well-tolerated in this high risk population, low discontinuation rate
  - All SAEs unrelated to study drug
- Overall, 6/122 patients experienced decompensation events through two years of treatment
  - 5/6 had either elevated baseline MELD and/or baseline platelets <100k

1. Deaths are Covid and metastatic cancer. 2. Common AE safety data extended beyond two years in some patients.

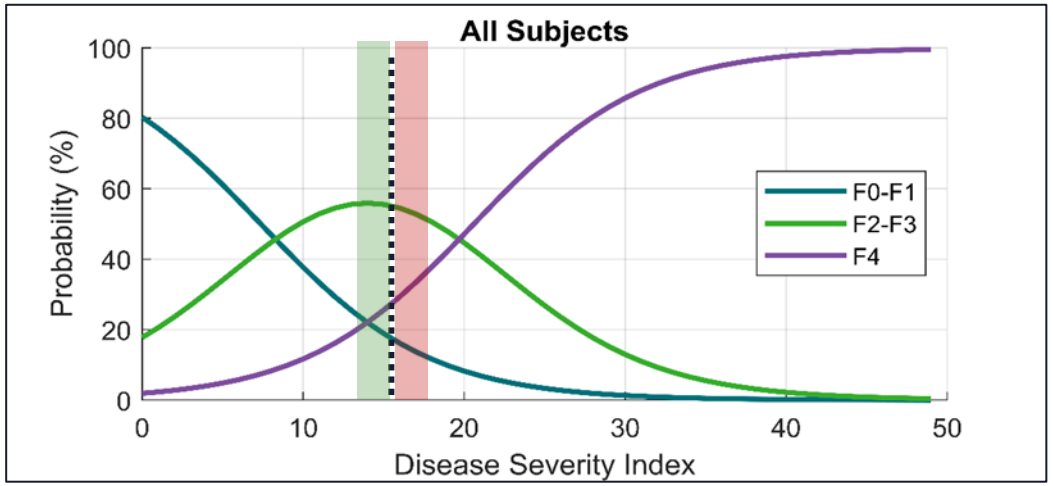
7%

Alkhouri N et al. J Hepatol 2025;82(S1):S9-S10. Presented at Late Breaker Session EASL ILC 2025.

DSI may Predict Reduction in Progression to Cirrhosis

Baseline DSI 16

Rx Efficacy | **Placebo - Progression**



	Probability		
DSI	**F0-F1**	**F2-F3**	**F4**
14	22.0%	55.9%	**22.1%**
15	18.9%	55.5%	25.6%
16	16.1%	54.5%	29.3%
17	13.7%	52.8%	33.5%
18	11.6%	50.6%	**37.8%**

Assume 1:1 (Rx : placebo)

Comparison of proportions (chi-square)

		Change in % with F4	
Case 1	Placebo progression over 2 years	8.5%	
	Rx arm stays the same		
	alpha=0.05, power 80%:	507 per arm (1014 total)	
	alpha=0.05, power 90%:	670 per arm (1340 total)	
Case 2	Rx improves DSI by 2	-7.3%	
	Placebo arm stays the same		
	alpha=0.05, power 80%:	605 per arm (1210 total)	
	alpha=0.05, power 90%:	800 per arm (1600 total)	
Case 3	Rx improves DSI by 2	15.7%	difference
	Placebo arm progresses by DSI 2		
	alpha=0.05, power 80%:	145 per arm (290 total)	
	alpha=0.05, power 90%:	190 per arm (380 total)	

* HepQuant Data on File – Histology from 455 liver biopsies with accompanying DSI measurements

Improving Design of Clinical Trials

Estimating Risk for Clinical Outcome from Baseline DSI

Baseline DSI	Estimated Clinical Outcome Rate (%)		
	1 year	2 years	5 years
20	3.3	8.2	17.9
21	3.9	9.6	20.8
22	4.6	11.3	24.1
23	5.4	13.2	27.8
24	6.3	15.4	31.9
25	7.4	17.9	36.5

From the RISK ACE model

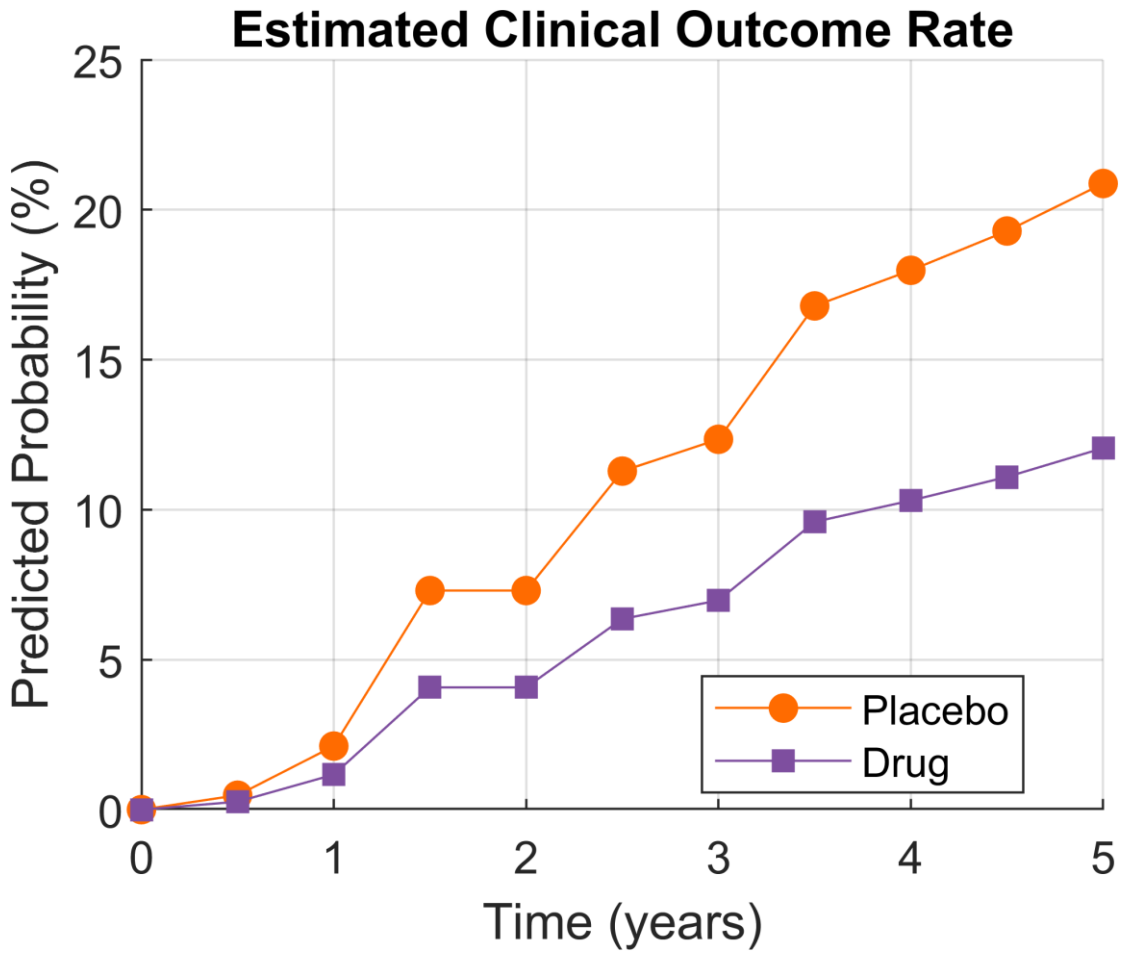
Aiding Study Design

Basic Inputs to Model

- Randomization ratio
- Baseline DSI of study population

- Assumptions for Estimating Sample Size
  - Placebo – Change in DSI
  - Anticipated Treatment Effect

Estimated rates of clinical outcome for placebo and treatment arms would be generated from HepQuant RISK ACE models (based on baseline, and baseline plus follow-up DSI).



From the RISK ACE model

Determining Sample Size

- Serial testing of 473 subjects with 1258 HepQuant DuO tests: SD of ΔDSI 3.38 (± 1.10)
- Serial testing of subjects in Placebo Arms: ΔDSI = -0.11 (± 1.02); SD of ΔDSI = (2.64 (± 1.02)

Sample Size Calculation (alpha 0.01, power 90%)			
Randomization 1:1; SD of ΔDSI for placebo 2.64 and for treatment 3.38			
Difference of Means			
	Difference in Mean DSIs	**per arm**	**total**
	1	276	552
	1.5	124	248
	2	71	142

HepQuant Data on File

Summary

Summary

- DSI and RISK ACE predict risk for clinical outcome – HALT-C study, Baylor Cirrhosis study, PSC study
- Stability in DSI or reduction in DSI with serial measurements is associated with reduced risk for clinical outcome (RISK ACE)
- Increase in DSI with serial measurements is associated with increased risk for clinical outcome (RISK ACE)
- Measurements of DSI, in both HCV (interferon/ribavirin and direct-acting antivirals) and MASH (Rezdiffra) treatment trials, not only have shown improvement in liver function and physiology but also that these effects are associated with reduced risk for clinical outcome – HALT-C, SOLAR-1, MAESTRO NAFLD1 OLE

Advantages of HepQuant DuO for the Clinic and Clinical Trials

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