Endpoints in Cirrhosis Trials (Peter T)

- Clinical decompensation (Survives): Ascites, Variceal hemorrhage, Encephalopathy, SBP, Liver-related Death, and, possibly HCC
- Patient reported outcomes (Feels)
- Liver Biopsy: Cirrhosis regression (1 stage drop in NASH CRN Fibrosis Stage)
- Prevention of development of varices (endoscopic endpoint)
- Reduction in MELD or CTP scores or other Cirrhosis scores
- Improvement in Liver Function
  - HepQuant SHUNT or STAT
  - MBT – showed data on this test result – moves in parallel with his HVPG data – p <0.03 in sub-analysis
HepQuant Tests for Clinical Trials

1 FDA issued Investigational Device Exemptions (IDEs)
## Ideal Diagnostic for Anti-Fibrotic & NASH Rx

- Reproducible
- Plausible link to pathogenesis of the disease
- Assess the whole organ
- Minimally invasive, well-tolerated
- Measure effectively all stages of disease but mainly exhibit accuracy in early stages
- Work in relevant populations (NASH, PSC)
- Standardized
- Apply across centers

## HepQuant Tests

- ✔ Reproducible
- ✔ Plausible link to pathogenesis of the disease
- ✔ Assess the whole organ
- ✔ Minimally invasive, well-tolerated
- ✔ Measure effectively all stages of disease but mainly exhibit accuracy in early stages
- ✔ Work in relevant populations (NASH, PSC)
- ✔ Standardized
- ✔ Apply across centers
HepQuant as Alternative to Invasive Standards and other Tests

HepQuant SHUNT links to clinical outcome; Alternative to liver biopsy, HVPG, and other tests.

HVPG is best surrogate for clinical outcomes from the group of competing technologies.

HepQuant SHUNT

Minimally-Invasive Alternative
HepQuant LLC and the HepQuant Tests
HepQuant’s Products: Test Kits

Supply Chain

- Manufacturer of stable isotope (cold)-labeled test compounds
- Central Pharmacy for formulation of test doses
- Assembly and shipping of Test Kits

Packaging: There is a 9 x 6 1/2 x 2 ¾” outer kit box that contains a foil pouch envelope, which contains an ambient gel wrap holding the interior kit contents (test solutions, other supplies). The kit boxes are shipped within a sealed cooler (either 2 or 5 kits per shipment) that is packaged within an outer shipping box.
The HepQuant Products

HepQuant SHUNT Liver Diagnostic Kit

HepQuant STAT Liver Diagnostic Kit
HepQuant’s Services: LC/MS Analyses

SHUNT and STAT Analyses

LC/MS Assay
- Minimal sample prep; rapid run times
- Standard Clinical LC/MS Equipment
  - Assay Validation
  - Method Transfer
Test Administration and Test Outputs
Test Administration

**SHUNT**

- Place Indwelling Intravenous catheter
- Oral (D4-cholate, 40 mg) and IV (13C-cholate, 20 mg)
- Timed blood draws at t = 5, 20, 45, 60 and 90 minutes
- Serum shipped for LC/MS analysis
- Measures HepQuant’s Disease Severity Index (**DSI**)

**STAT**

- No Indwelling catheter
- Oral (D4-cholate, 40 mg) only
- Single blood draw at t = 60 minutes
- Serum shipped for LC/MS analysis
- Estimates **DSI**
Test Outputs

**SHUNT**

- Phase 1 \( k_{\text{elim}} \) and \( V_d \)
- Clearance from total inflow (Systemic HFR)
- Clearance from portal inflow (Portal HFR)
- Portal-systemic spillover (SHUNT)
- Measures Disease Severity Index (DSI)

**STAT**

- Concentration D4-CA at 60 min - STAT
- Estimates Disease Severity Index (DSI)
Relationship of STAT to DSI

Clinical Stage of Disease       None  Mild  Port Hyp  Decompensation  End-Stage Disease

N = 1010 Tests in ~500 patients
HCV
PSC
NAFLD
DSI Quantifies

Hepatocyte uptake of cholate

Hepatic perfusion from systemic circulation
Hepatic perfusion from portal circulation
Portal-systemic shunting (spillover)

DSI is the minimally invasive alternative to HVPG
Reproducibility
Reproducibility

**DSI**
- Study 1, Triplicates $iCC = 0.931$
- Study 2, Duplicates $iCC = 0.948$

**STAT**
- Study 1, Triplicates $iCC = 0.894$
- Study 2, Duplicates $iCC = 0.918$
Link to Pathobiology of Liver Disease
B

Normal

Stellate cell
Sinusoid
Endothelial cell

Injured

Stellate cell
Endothelial cell

Balanced vasoconstrictors and dilators

Endothelial cell
Normal sinusoids
Stellate cell
Endothelial cell fenestrae

Imbalance in vasoconstrictors and dilators

Endothelial cell
Abnormal sinusoids
Activated stellate cell

Increased:
- PDGF → HSC migration
- Endothelin → Vasoconstriction, HSC activation and contractibility
- TGFβ → HSC activation, fibrogenesis
- VEGF → Angiogenesis permeability

Decreased:
- NO → Vasoconstriction, HSC activation
Liver Disease Alters Liver Cell Function and the Portal Circulation

- Impaired Sinusoidal Perfusion
- Portal Hypertension
- Collaterals
- Varices
- Poor Function + Portal Hypertension Collaterals
  - Increases Spillover (HQ SHUNT)
HepQuant Measures the Altered Liver Cell Function and Portal Circulation

Decreased HFRs (Clearances)  
Increased Portal-systemic SHUNting  
↓ Portal HFR  
↓ Systemic HFR  
Worsening DSI  
Port HFR < Syst HFR  
↑ SHUNT
Quantifying Severity of Liver Disease
Both STAT and DSI Correlate with Fibrosis and Predict Risk of Outcomes

Pearson Correlations are using all data points. Symbols are the mean ± SD. Optimum Cutoffs for High Risk of Outcomes are STAT ≥ 1.12 µM and DSI ≥ 18.63
Predict Risk for Clinical Outcome: HALT-C

**DSI**

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>n=52</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTP +2</td>
<td>18</td>
</tr>
<tr>
<td>Var Bleed</td>
<td>4</td>
</tr>
<tr>
<td>Ascites</td>
<td>8</td>
</tr>
<tr>
<td>Enceph</td>
<td>3</td>
</tr>
<tr>
<td>Asc+Enc</td>
<td>3</td>
</tr>
<tr>
<td>Death</td>
<td>16</td>
</tr>
</tbody>
</table>

**STAT**

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>n=52</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTP +2</td>
<td>18</td>
</tr>
<tr>
<td>Var Bleed</td>
<td>4</td>
</tr>
<tr>
<td>Ascites</td>
<td>8</td>
</tr>
<tr>
<td>Enceph</td>
<td>3</td>
</tr>
<tr>
<td>Asc+Enc</td>
<td>3</td>
</tr>
<tr>
<td>Death</td>
<td>16</td>
</tr>
</tbody>
</table>
# Independent and Strongest Predictors for Clinical Outcome

## DSI

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSI tertile 15.395-19.898</td>
<td>2.40</td>
<td>0.64</td>
<td>9.04</td>
<td>0.196</td>
</tr>
<tr>
<td>DSI tertile &gt;19.898</td>
<td>14.01</td>
<td>3.84</td>
<td>51.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrosis ISHAK 5,6 vs 2,3,4</td>
<td>1.15</td>
<td>0.52</td>
<td>2.54</td>
<td>0.730</td>
</tr>
<tr>
<td>Platelets per unit</td>
<td>0.99</td>
<td>0.99</td>
<td>1.00</td>
<td>0.117</td>
</tr>
<tr>
<td>Age per year</td>
<td>0.98</td>
<td>0.94</td>
<td>1.02</td>
<td>0.300</td>
</tr>
<tr>
<td>Gender Male vs Female</td>
<td>1.23</td>
<td>0.64</td>
<td>2.38</td>
<td>0.538</td>
</tr>
<tr>
<td>Race Black vs Non-Hispanic, White</td>
<td>0.48</td>
<td>0.18</td>
<td>1.26</td>
<td>0.136</td>
</tr>
<tr>
<td>Race Hispanic/other vs Non-Hispanic, White</td>
<td>0.97</td>
<td>0.47</td>
<td>2.00</td>
<td>0.940</td>
</tr>
</tbody>
</table>

## STAT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAT tertile 0.73-1.19</td>
<td>2.59</td>
<td>0.70</td>
<td>9.56</td>
<td>0.154</td>
</tr>
<tr>
<td>STAT tertile &gt;1.19</td>
<td>9.82</td>
<td>2.82</td>
<td>34.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrosis ISHAK 5,6 vs 2,3,4</td>
<td>1.58</td>
<td>0.79</td>
<td>3.17</td>
<td>0.199</td>
</tr>
<tr>
<td>Platelets per unit</td>
<td>0.99</td>
<td>0.99</td>
<td>1.00</td>
<td>0.059</td>
</tr>
<tr>
<td>Age per year</td>
<td>0.98</td>
<td>0.94</td>
<td>1.02</td>
<td>0.263</td>
</tr>
<tr>
<td>Gender Male vs Female</td>
<td>1.10</td>
<td>0.58</td>
<td>2.08</td>
<td>0.771</td>
</tr>
<tr>
<td>Race Black vs Non-Hispanic, White</td>
<td>0.69</td>
<td>0.26</td>
<td>1.81</td>
<td>0.451</td>
</tr>
<tr>
<td>Race Hispanic/other vs Non-Hispanic, White</td>
<td>1.01</td>
<td>0.49</td>
<td>2.05</td>
<td>0.987</td>
</tr>
</tbody>
</table>
Compensated Cirrhosis: Distinguishing High- from Low-Risk
Child Class A Cirrhotic Patients

**Decomp-free Survival by DSI 20**
- DSI<20 (40%)
- DSI>20 (60%)
- P < 0.001

**Decomp-free Survival by STAT 0.9 µM**
- STAT<0.9 (23%)
- STAT>0.9 (77%)
- P < 0.001
DSI and STAT in NASH
HepQuant Tests: Identifying NASH Subjects

Results: Identifying Potential Subjects with NASH

50 healthy controls (including normal weight, overweight, and obese) and 39 biopsied NASH patients were tested. The optimum cutoff on the DSI ROC was set by Youden Index. The cutoff on the STAT ROC was set at the same Specificity.

HepQuant®-SHUNT Test

DSI > 16.5

DSS
Sens. Spec.
87% 98%

PPV NPV
97% 91%

DSI ROC Curve

AUC c-statistic = 0.96

The high Specificity (98%) and the high PPV (97%) at the optimum cutoff (DSI >16.5) indicate that almost all of the potential subjects above the cutoff will likely have biopsy-proven NASH. The high Sensitivity (87%) means few NASH patients will be missed.

HepQuant®-STAT Test

STAT > 0.65 μM

Sens. Spec.
69% 98%

NPV
96% 80%

STAT ROC Curve

AUC c-statistic = 0.94

The cutoff (STAT >0.65 μM) was chosen to maintain the high Specificity (98%) and this keeps a high PPV (96%). Almost all of the potential subjects above the cutoff will likely have biopsy-proven NASH, but reduced Sensitivity (69%) yields fewer patients.

Poster Session: April 24, 2018; Steve Helmke PhD 1st Author
HepQuant Tests: Identifying NASH F3 + Compensated F4

Results: Identifying Potential Subjects with NASH F3/Compensated F4
The NASH cohort included 8 patients with early stage fibrosis (F1/F2), 9 patients with advanced fibrosis (F3), 13 patients with compensated cirrhosis (CompF4), and 9 patients with decompensated cirrhosis (DecompF4). The optimum ranges for DSI (17.2 – 30.0) and STAT (0.81 – 2.5 μM) to select F3/CompF4 NASH patients were set by ROC curve Youden Indices. The distribution of the NASH patients by histological staging and by DSI and STAT ranges are shown in the tables below.

<table>
<thead>
<tr>
<th>Histology</th>
<th>DSI &lt; 17.2</th>
<th>17.2 &lt; DSI &lt; 30.0</th>
<th>DSI &gt; 30.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decompensated Cirrhosis (F4)</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Compensated Cirrhosis (F4)</td>
<td>3</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Advanced Fibrosis (F3)</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Most of the potential subjects in the DSI range (PPV 86%) will be F3/CompF4. The high Sensitivity (82%) means few NASH F3/CompF4 patients will be missed.

<table>
<thead>
<tr>
<th>Histology</th>
<th>STAT &lt; 0.81</th>
<th>0.81 &lt; STAT &lt; 2.5</th>
<th>STAT &gt; 2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decompensated Cirrhosis (F4)</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Compensated Cirrhosis (F4)</td>
<td>9</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Advanced Fibrosis (F3)</td>
<td>9</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Early Fibrosis (F1-2)</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Most of the potential subjects in the STAT range (PPV 87%) will be F3/CompF4. The reduced Sensitivity (59%) will yield fewer of the patients with those stages.

Poster Session: April 24, 2018; Steve Helmke PhD 1st Author
Risk for Varices: Comparing NASH and HCV
## DSI

| A. Ability to Diagnose NASH in a cohort of Healthy Controls (N=50) and 31 NASH Patients (N=31) |
|---------------------------------|-----------------|-----------------|-------|-------|-------|-------|-----------|
| HQ-SHUNT                        | AUROC c-statistic | optimum cutoff | Sensitivity | Specificity | PPV   | NPV   | Youden Index (J) |
|                                 | 0.94             | DSI > 16.5      | 84%         | 98%         | 96%   | 91%   | 0.82      |

| B. Ability to Identify Patients who had Medium/Large Varices (N=9) in the NASH cohort (N=31) |
|---------------------------------|-----------------|-----------------|-------|-------|-------|-------|-----------|
| HQ-SHUNT                        | AUROC c-statistic | optimum cutoff | Sensitivity | Specificity | PPV   | NPV   | Youden Index (J) |
|                                 | 0.92             | DSI > 28        | 89%         | 91%         | 80%   | 95%   | 0.80      |
| Biopsy                          | 0.80             | Cirrhosis       | 100%        | 59%         | 50%   | 100%  | 0.59      |

| C. Ability to Identify Patients who had Decompensation (N=9) in the NASH cohort (N=31) |
|---------------------------------|-----------------|-----------------|-------|-------|-------|-------|-----------|
| HQ-SHUNT                        | AUROC c-statistic | optimum cutoff | Sensitivity | Specificity | PPV   | NPV   | Youden Index (J) |
|                                 | 0.99             | DSI > 28        | 100%       | 95%         | 90%   | 100%  | 0.95      |
| Biopsy                          | 0.80             | Cirrhosis       | 100%        | 59%         | 50%   | 100%  | 0.59      |

## STAT

<table>
<thead>
<tr>
<th>Diagnosing NASH</th>
<th>AUROC c-statistic</th>
<th>optimum cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Youden Index (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosing NASH Var</td>
<td>0.93</td>
<td>STAT &gt; 0.50 μM</td>
<td>94%</td>
<td>76%</td>
<td>71%</td>
<td>95%</td>
<td>0.70</td>
</tr>
<tr>
<td>Diagnosing NASH Cirrhosis</td>
<td>0.90</td>
<td>STAT &gt; 1.08 μM</td>
<td>86%</td>
<td>88%</td>
<td>86%</td>
<td>88%</td>
<td>0.74</td>
</tr>
<tr>
<td>Diagnosing NASH Lg Var</td>
<td>0.86</td>
<td>STAT &gt; 1.25 μM</td>
<td>67%</td>
<td>100%</td>
<td>100%</td>
<td>68%</td>
<td>0.67</td>
</tr>
<tr>
<td>Diagnosing NASH Var</td>
<td>0.93</td>
<td>STAT &gt; 2.40 μM</td>
<td>89%</td>
<td>95%</td>
<td>89%</td>
<td>95%</td>
<td>0.84</td>
</tr>
</tbody>
</table>
Correlation with Portal Pressure
Portal-systemic Shunt Fraction measured by HepQuant-SHUNT Test Correlates with the Hepatic Venous Pressure Gradient (HVPG)

SHUNT showed a strong and highly significant positive correlation with both dPP and IF (r=0.65, p = 0.0005 and r=0.75, p = <0.0001, respectively). Additionally, patients with severe fibrosis demonstrated significantly higher degrees of SHUNT compared to those with mild disease (p<0.0001).

SHUNT correlated with portal and peripheral sCD163 (r=0.48, p=0.01, r=0.47, p=0.01 respectively) and portal inflammatory markers such as IFNγ, IL6, IL8 and TNFα (r=0.44, p=0.02, r=0.49, p=0.009, r=0.60, p=0.0007, r=0.52, p=0.005).

DCT (Dual Cholate Test – tradename is HepQuant SHUNT Test) correlates with established markers of disease severity across the spectrum of LD. DCT may provide insights into the biology of LD by exploration of associations with SHUNT and HFR. These findings encourage further exploration of the utility of DCT as a non-invasive tool for stratification and management of patients with chronic LD.

Liver Functional Assessment by Dual Cholate Testing Is Strongly Correlated with Direct Portal Pressure and Ishak Fibrosis Stage
DSI for Measuring Treatment Effects
The HepQuant SHUNT Test

**Administration**

- Peripheral venous catheter
- Oral (D4-cholate, 40 mg)
- Simultaneously, IV (13C-cholate, 20 mg)
- Blood samples at t = 5, 20, 45, 60 and 90 minutes
- Quantifies HFRs, SHUNT, and DSI

**Interpretation**

<table>
<thead>
<tr>
<th>% of Max Hepatic Capacity</th>
<th>None</th>
<th>Mild</th>
<th>Varices</th>
<th>Decomp</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpretation</td>
<td>Better</td>
<td>Worse</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hepatic Disease Risk

- None
- Mild
- Varices
- Decomp
- Death

DSI

![Graph showing % of Max Hepatic Capacity vs. Hepatic Disease Risk]

- Better
- Worse
DSI* for Monitoring Treatment Effects

Baseline DSI: DSI at t0.  \( \Delta \text{DSI}: [\text{DSI at } t_i - \text{DSI at } t_0] \)

* DSI is calculated from Portal and Systemic Hepatic Filtration Rates (HFRs) as measured by HepQuant SHUNT.
ΔDSI with No Treatment: “Placebo Arm”
Measuring Disease Severity and Progression
(137 Patients with DSI and ISHAK Fibrosis Scores F2 – F5 at Baseline and 2 Yr)

<table>
<thead>
<tr>
<th></th>
<th>NonProgressors</th>
<th>Fibrosis Progressors</th>
<th>Progress to Cirrhosis</th>
<th>Experience Clinical Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSI</td>
<td>95</td>
<td>42</td>
<td>33</td>
<td>19</td>
</tr>
</tbody>
</table>

- Baseline DSI
- ΔDSI (Yr 2 - Base)
<table>
<thead>
<tr>
<th></th>
<th>DSI</th>
<th>Alkaline Phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (V1)</td>
<td>SD</td>
</tr>
<tr>
<td>V1</td>
<td>17.67</td>
<td>7.38</td>
</tr>
<tr>
<td>V2</td>
<td>17.81</td>
<td>7.08</td>
</tr>
<tr>
<td>Avg V2-V1</td>
<td>0.14</td>
<td>2.99</td>
</tr>
<tr>
<td>Avg Base</td>
<td>17.74</td>
<td>7.07</td>
</tr>
<tr>
<td>V3</td>
<td>18.88</td>
<td>7.86</td>
</tr>
</tbody>
</table>

\[ \Delta \text{DSI Avg Base to V3} \quad 1.14 \quad 3.91 \quad 0.0363 \]

V1 and V2 were two baseline tests done within 15.6 ± 9.7 days of each other; V3 was done 393 ± 37 days after baseline. There was no significant difference in Baseline DSI between V1 and V2 (p = 0.3808). The increase in DSI from Baseline to V3 was statistically significant (p=0.0363). The increases in DSI from V1 to V3, or V2 to V3 were also statistically significant. Importantly, in comparison, there was no significant change in Alk Phos.
DSI in Detecting Treatment Effects
### ΔDSI in Diverse Cohorts

<table>
<thead>
<tr>
<th>Group</th>
<th>N, Patients</th>
<th>TimeFrame</th>
<th>Mean ΔDSI</th>
<th>SD of ΔDSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTx F0 – F3</td>
<td>10</td>
<td>4 Weeks</td>
<td>-3.7</td>
<td>3.9</td>
</tr>
<tr>
<td>MELD ≤10</td>
<td>4</td>
<td>36 Weeks</td>
<td>-4.4</td>
<td>3.4</td>
</tr>
<tr>
<td>HALT-C SVR</td>
<td>24</td>
<td>2 Years</td>
<td>-2.1</td>
<td>2.9</td>
</tr>
<tr>
<td>LTx Cirrhosis</td>
<td>11</td>
<td>24 Weeks</td>
<td>-3.7</td>
<td>3.2</td>
</tr>
<tr>
<td>CP-B</td>
<td>8</td>
<td>60 Weeks</td>
<td>-4.5</td>
<td>2.6</td>
</tr>
<tr>
<td>Decomp Cirrh</td>
<td>10</td>
<td>4 Weeks</td>
<td>0.2</td>
<td>2.8</td>
</tr>
<tr>
<td>HALT-C No Rx</td>
<td>97</td>
<td>2 Years</td>
<td>2.1</td>
<td>5.0*</td>
</tr>
<tr>
<td>HALT-C NR</td>
<td>91</td>
<td>2 Years</td>
<td>1.1</td>
<td>5.5*</td>
</tr>
<tr>
<td>PSC Natl Hx</td>
<td>46</td>
<td>1 Year</td>
<td>0.7</td>
<td>3.5</td>
</tr>
</tbody>
</table>

In HALT-C, there was more variation in ΔDSI in the untreated or nonresponder patients compared to patients achieving SVR. This was likely due to the interval between baseline and followup DSI measurements of 2 years – the results are consistent with the expected variation in disease progression between these groups of patients.
Use of DSI in SOLAR-1 is relevant to shorter term clinical trials – DSI was measured 5 times in 31 patients over 48 weeks. The patients had a spectrum of liver disease ranging from liver transplant recipients with recurrent hepatitis C with fibrosis to non-transplant patients with decompensated CTP B or C cirrhosis. The $\Delta$DSIs (±SD) for change between time points are:

<table>
<thead>
<tr>
<th>Timeframe</th>
<th>N</th>
<th>Mean $\Delta$DSI</th>
<th>Std Dev of $\Delta$DSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 4 – Base</td>
<td>28</td>
<td>-2.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Week 24 – Base</td>
<td>27</td>
<td>-2.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Week 36 – Base</td>
<td>29</td>
<td>-1.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Week 48 – Base</td>
<td>28</td>
<td>-0.8</td>
<td>3.7</td>
</tr>
</tbody>
</table>

A SD of 4 for $\Delta$DSI is a conservative estimate for use in study design to determine sample sizes.
Detecting Rx Effect using SD 4 for ΔDSI

<table>
<thead>
<tr>
<th>Comparators</th>
<th>Mean ΔDSI</th>
<th>SD of ΔDSI</th>
<th>N 80% Power</th>
<th>N 90% Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base vs Post-Rx</td>
<td>1</td>
<td>4</td>
<td>128</td>
<td>171</td>
</tr>
<tr>
<td>Base vs Post-Rx</td>
<td>2</td>
<td>4</td>
<td>34</td>
<td>44</td>
</tr>
<tr>
<td>Base vs Post-Rx</td>
<td>3</td>
<td>4</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Base vs Post-Rx</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>13</td>
</tr>
</tbody>
</table>

This is for a single arm trial using paired analysis – comparing baseline results with results at subsequent, post-Rx timepoints. The proposed study of 45 is sufficient to detect a change in DSI of 2 or more. A smaller change in DSI will be detected if the SD of ΔDSI is <4.
Tolerability
Tolerability of Dual Cholate Testing (Serial Testing)

The HepQuant SHUNT test is well tolerated. In a substudy of the SOLAR-1 trial of DAA therapy of HCV, 31 patients underwent five HepQuant SHUNT tests serially over 48 weeks (50,52,53). All 31 patients completed the survey of their experience with the HepQuant SHUNT test after a mean (±SD) of 4.5±0.6 tests (Appendix D). Pain was rated 0.5±0.8, where 0 was no pain and 10 was severe pain. Discomfort was rated 0.3±0.5, where 0 was no discomfort and 10 was severe discomfort. Inconvenience was rated 0.4±0.9, where 0 was no interference with daily activity and 10 was complete inability to conduct usual daily activity. The recording of time commitment indicated <3 hours in 23 cases and >3 h in 8 cases. Overall experience with the test was rated 9.5±1.1, where 0 was very negative and 10 was very positive. Willingness to undergo additional testing was rated 9.1 ±1.8, where 0 was definitely not willing and 10 was very willing.
Tolerability of Dual Cholate Testing (vs HVPG)

In another study of 20 patients we compared the tolerability of a single HepQuant SHUNT test to the tolerability of HVPG (54). The results for the HepQuant SHUNT test were very similar to those observed in the SOLAR-1 study. Pain was rated 0.4±0.6, where 0 was no pain and 10 was severe pain. Discomfort was rated 0.5±0.9, where 0 was no discomfort and 10 was severe discomfort. Inconvenience was rated 1.25±1.41, where 0 was no interference with daily activity and 10 was complete inability to conduct usual daily activity. The recording of time commitment indicated <3 hours in 18 of 20 cases and 3 to 6 h in 2 cases. No patient recorded >6 h of time commitment. Overall experience with the test was rated 9.6±0.7, where 0 was very negative and 10 was very positive. Willingness to undergo additional testing was rated 9.9±0.4, where 0 was definitely not willing and 10 was very willing.

In contrast, HVPG was far less tolerable and acceptance highly variable (54). Using the same survey of patient reported outcomes, pain was rated 3.9±3.1, discomfort 4.2±2.9, and inconvenience 5.9±3.1. The recording of time commitment indicated 0 to 3 hours in 4, 3 to 6 h in 5, 6 to 9 h in 3, and over 9 h in 8. Overall experience with HVPG was rated 6.2±2.7, and willingness to undergo additional testing was rated 5.7±3.6.

Not surprisingly, the minimally-invasive HepQuant SHUNT test was much better tolerated and had higher patient acceptance than the invasive HVPG measurement.
Logistics of Performing HepQuant SHUNT in the Trial
HepQuant SHUNT: Facility-based Test

Decision to test liver with HepQuant SHUNT

Patient goes to testing center

Blood collected and sent to lab

Results sent to provider

Lab Analysis

Care Provider Orders Test

HepQuant LLC ships kits to Testing Centers

HepQuant Test Kits

Review Results (DSI Score) with Patient
HepQuant STAT: Point-of-Care Test

HepQuant LLC ships kits to Provider Office

HepQuant STAT performed in Office

Blood collected and sent to lab

Lab Analysis

Results sent to provider

Review Results (DSI Score) with Patient

HepQuant Test Kits
Ideal Diagnostic for Anti-Fibrotic & NASH Rx

- Reproducible
- Plausible link to pathogenesis of the disease
- Assess the whole organ
- Minimally invasive, well-tolerated
- Measure effectively all stages of disease but mainly exhibit accuracy in early stages
- Work in relevant populations (NASH, PSC)
- Standardized
- Apply across centers

HepQuant Tests

- ✓ Reproducible
- ✓ Plausible link to pathogenesis of the disease
- ✓ Assess the whole organ
- ✓ Minimally invasive, well-tolerated
- ✓ Measure effectively all stages of disease but mainly exhibit accuracy in early stages
- ✓ Work in relevant populations (NASH, PSC)
- ✓ Standardized
- ✓ Apply across centers
Function replace Fibrosis? Or, Stiffness?

“No Way!”
“You’re dreaming!”
“You’re crazy!”
<table>
<thead>
<tr>
<th>No.</th>
<th>Quote</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>“If excessive smoking actually plays a role in the production of lung cancer, it seems to be a minor one.”</td>
<td>W.C. Hesper, National Cancer Institute, 1954</td>
</tr>
<tr>
<td>16</td>
<td>“Television won’t last because people will soon get tired of staring at a plywood box every night.”</td>
<td>Darryl Zanuck, movie producer, 20th Century Fox, 1946</td>
</tr>
<tr>
<td>17</td>
<td>“The horse is here to stay but the automobile is only a novelty—a fad.”</td>
<td>The president of the Michigan Savings Bank advising Henry Ford's lawyer not to invest in the Ford Motor Co., 1903</td>
</tr>
<tr>
<td>18</td>
<td>“Everyone acquainted with the subject will recognize it as a conspicuous failure.”</td>
<td>Henry Morton, president of the Stevens Institute of Technology, on Edison’s light bulb, 1880</td>
</tr>
<tr>
<td>19</td>
<td>“X-rays will prove to be a hoax.”</td>
<td>Lord Kelvin, President of the Royal Society, 1883</td>
</tr>
<tr>
<td>20</td>
<td>“I think there is a world market for maybe five computers.”</td>
<td>Thomas Watson, chairman of IBM, 1943</td>
</tr>
<tr>
<td>23</td>
<td>“This ‘telephone’ has too many shortcomings to be seriously considered as a means of communication. The device is inherently of no value to us.”</td>
<td>Western Union internal memo, 1876</td>
</tr>
<tr>
<td>24</td>
<td>“We don’t like their sound, and guitar music is on the way out.”</td>
<td>Decca Recording Company on declining to sign the Beatles, 1962</td>
</tr>
</tbody>
</table>
HepQuant Tests in Liver Disease Management

**Who are these folks?**

- **Fatty Liver > NASH > Cirrhosis**
- Alcohol > Fat > Cirrhosis
- HBV > hepatitis > Cirrhosis
- HCV > hepatitis > Cirrhosis
- Autoimmune > Cirrhosis
- Genetic > Cirrhosis

**30 M At Risk for CLD**

- **8.5 M With CLD**
  - STAT Screen

- **1 M With F3/F4 CLD**
  - SHUNT

- **1 M F3/F4**
  - Varices Monitoring Rx

- **21.5 M For Follow-Up**
  - Repeat **STAT**

- **7.5 M For Follow-Up**
  - Repeat **STAT**

- **Repeat SHUNT for Rx Effect**

**HepQuant Tests in Liver Disease Management**

- **SHUNT**
- **Varices Monitoring Rx**
- **Repeat SHUNT for Rx Effect**

---

**Screen**

**Follow-Up**

**Repeat STAT**

**STAT**