Measuring the Liver’s Health

Linking Function to Clinical Outcomes

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Director of Hepatology
University of Colorado Denver
Function-”omics”
Disclosures

• Intellectual property filings with University of Colorado Denver – 6 patents have been issued from 12/2013 to 7/2015; others pending.

• Founder, Manager and Equity Member of HepQuant LLC

The Dual Cholate tests discussed in this presentation are NOT FDA APPROVED. They are currently for investigational use only.
Functional Assessment as an Alternative to Fibrosis Measurement

Functional impairment, not fibrosis, is the direct link to clinical outcome.
Ideal Traits for a Test of Liver’s Health

- Minimally invasive, well tolerated
- Reproducible
- Plausibly linked to pathogenesis of the disease
- Assess the whole organ
- Measure effectively all stages of disease— including early stages
- Intended for use in relevant populations (HCV, NASH, PSC, Chronic Liver Disease)
- Standardized test administration and lab analysis
- Applied across multiple centers
- Detect Treatment Effects
- High Value
Minimally Invasive
Paradigm shift toward Noninvasive Alternatives

Fibrosis Stage on liver biopsy as the Invasive “Gold Standard”

Measuring Function and Physiology

Dual Cholate SHUNT

Estimating Fibrosis

Liver Stiffness

Metabolism

Breath Tests

SPECT scan

Biomarkers
Our HEPATIQ™ software provides a precise index of liver disease severity. The HEPATIQ™ technology automates the Quantitative Liver Spleen Scan (QLSS™) that has been proven to be an accurate predictor of clinical outcomes in the recently concluded HALT-C trial.

The HEPATIQ Software has been cleared by the Food and Drug Administration for sale in the U.S.A.


13C-Methacetin BT (Breath ID)

Results:
The cumulative percent dose recovery 20 minutes after methacetin ingestion (CPDR20) correlated with the degree of disease severity with $r^2 = 0.62$ (p<0.0001).

- Metabolic reserve by MBT was maintained in pre-cirrhotic NASH, with 15.5% reduction in mean metabolic function compared to healthy controls.
- The mean metabolic function markedly declined between pre-cirrhotic NASH and compensated cirrhosis as well as between compensated cirrhosis and advanced NASH cirrhosis with a 27.4% and 72.3% reduction in CPDR20, respectively.
- MBT differentiated early NASH from compensated cirrhosis and advanced NASH cirrhosis (AUC=0.84, 95% CI: 0.78-0.89; p<0.0001).

Poster available for viewing – NASH Biomarker Workshop Alexandria VA
MELD

13C- Methacetin BT

HepQuant SHUNT (DSI)

- Peripheral venous indwelling catheter
- Oral (D4-cholate, 40 mg) and IV (13C-cholate, 20 mg)
- Timed blood draws at t = 5, 20, 45, 60 and 90 minutes
- Quantifies HFRs, SHUNT, and DSI
Model for Decline in Dual Cholate Clearances with Disease Severity

Disease Progression (from Healthy (F0) to Severe Decomp (C5))

Reproducibility
## Reproducibility Studies: Subject Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>HCV</td>
</tr>
<tr>
<td>N, %, or Mean (± Standard Deviation)</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>32.7±11.6</td>
<td>55.1±6.6</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>8:8</td>
<td>13:3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9±2.0</td>
<td>28.3±4.1</td>
</tr>
<tr>
<td>Fibrosis Stage, N Pts (F0:F1:F2:F3:F4)¹</td>
<td>NA</td>
<td>3:2:3:4:4</td>
</tr>
<tr>
<td>Fibrosis Score¹</td>
<td>NA</td>
<td>2.3±1.4</td>
</tr>
<tr>
<td>MELD score</td>
<td>NA</td>
<td>7.6±1.5</td>
</tr>
<tr>
<td>CTP score</td>
<td>NA</td>
<td>5.2±0.5</td>
</tr>
<tr>
<td>Varices</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Ascites</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.7±0.2</td>
<td>1.1±0.4</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.0±0.3</td>
<td>3.9±0.4</td>
</tr>
<tr>
<td>INR</td>
<td>1.0±0.1</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.0±0.1</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Platelet Count (µL⁻¹)</td>
<td>243±46</td>
<td>152±66</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>14.3±1.4</td>
<td>15.1±1.8</td>
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</tbody>
</table>

¹ Fibrosis Stage: 0 = No fibrosis, 1 = Early fibrosis, 2 = Intermediate fibrosis, 3 = Advanced fibrosis, 4 = Cirrhosis
² ND = Not determined

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**HEPAQUANT**

**FUNCTION MATTERS**
Disease Severity Index (DSI)

Repeated Measures of DSI vs Mean DSI

\[ R^2 = 0.9500 \]
HepQuant STAT

- Oral dose only – D4 cholate 40 mg
- One blood draw at 60 minutes
- Serum separated and shipped to HQ lab
- STAT, estimated DSI, reported
Relationship of STAT to DSI

N = 1010 Tests in ~500 patients
HCV
PSC
NAFLD
Test Outputs

• Systemic Hepatic Filtration Rate (HFR)
• Portal HFR
• SHUNT
• Disease Severity Index (DSI)
• STAT

* DSI is a function of HFRs and SHUNT
Why Cholate?

Why Dual Label in the case of HepQuant SHUNT?
Cholate is Stable
Bile acids of a 3200-year-old Egyptian mummy.
Kuksis A, Child P, Myher JJ, Marai L, Yousef IM, Lewin PK.

Abstract
The bile acids of the gall bladder and hepatic tissue of a 3200-year-old Egyptian mummy were isolated by thin-layer chromatography and identified by combined gas-liquid chromatography and mass spectrometry. Except for complete deconjugation and extensive dehydration, the bile acids were found to correspond in their qualitative and quantitative composition to the gall bladder bile acids of modern man. The secondary bile acids constituted about 50% of the total and were identified as the normal bacterial oxidoreduction products of the primary bile acids and their dehydration products. In addition a series of unsaturated bile acids were identified, which corresponded to the dehydration products of cholic and chenodeoxycholic acids. It is suggested that both bile acid deconjugation and the limited oxidoreduction were probably brought about by the Clostridium organisms identified in the tissue. On the basis of the bile acid composition it is concluded that the ancient man metabolized cholesterol along the same pathways as modern man.
Cholates as Stable Probes

- Endogenous – primary bile acid of man
- Transport, disposition, metabolism well characterized
- No DDIs
- $^{13}\text{C, D} –$ stable, cold – ideal for multiple test admin
- No pharmacologic effect in the doses administered in any of the HepQuant tests (mg doses)
- $^{13}\text{C}$-cholate and $^{4}\text{D}$-cholate are molecular probes that can track cholate within the human body safely and noninvasively (nM accuracy)
- Blood test – no need for special equipment
Plausibly Linked to Pathogenesis
Elements of Functional Impairment

Healthy Liver

- Hepatic Vein
- Hepatic Artery
- Systemic Blood Compounds
- Portal Vein
- Portal Blood Compounds

Diseased Liver

- Hepatic Vein
- Hepatic Artery
- Portal Vein
- Portal Blood Compounds
- Systemic Blood Compounds

Factors:
- Viruses
- EtOH
- Auto-Immune
- Biliary
- Other

Shunt
Cholic Acid (CA) Transporters

- Portal-Systemic Shunting
  - Portal Vein
    - HEPATOCYTES
      - NTCP
      - BAAT
    - Hepatic Artery
      - Intravenous 13C-CHOLATE
- Enterocytes
  - OATP1B1
  - OATP1A2
  - OATP1B3
  - OST-α/β
  - MRP3
  - IBABP
  - ASBT
  - OATP1A2
- Oral 4D-CHOLATE
  - Stomach
  - Small Intestine
  - Colon
Assesses the Whole Organ
And
All Stages of Disease
Model for Decline in Dual Cholate Clearances with Disease Severity

Disease Progression (from Healthy (F0) to Severe Decomp (C5))

HCV
HALT-C Trial
Both STAT and SHUNT 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
DSI in HALT-C Cohort: Baseline DSI Predicts Risk for Clinical Outcome

Mean Period of Followup 5.5 years, up to 8.3 years. N = 220 patients, clinically compensated, with Ishak F2 to F6.

DSI v3.4

Selecting Optimum Cutoff - Youden Index

DSI cutoff 18.6

Sensitivity 87%
Specificity 72%
PPV 49%
NPV 95%
SHUNT (DSI) Predicts Risk for Clinical Outcome

With Number of Subjects at Risk and 95% Confidence Limits

<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>
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</table>
STAT Predicts Risk for Clinical Outcome

With Number of Subjects at Risk and 95% Confidence Limits

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Count</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>
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<td>Enceph</td>
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<tr>
<td>Asc+Enc</td>
<td>3</td>
</tr>
<tr>
<td>Death</td>
<td>16</td>
</tr>
</tbody>
</table>
# Function vs Fibrosis in Predicting Outcomes

## Ishak Fibrosis Stage

<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>Fibrosis ISHAK 5,6 vs 2,3,4</td>
<td>4.00</td>
<td>2.15</td>
<td>7.44</td>
<td>&lt;0.001</td>
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</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>
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<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>

## 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>
Decline of Hepatic Functional Reserve

\[ y = -5.9065x + 99.431 \]
\[ R^2 = 0.9842 \]

Stage of Disease

- Healthy Persons
- F0-F1
- F2
- F3
- F3-F4
- F4
- F5
- F6
- F5-F6
- Post-LT mix of CTP A B C
- Decompensated CTP B C
- Decompensated Waiting List Pts
- Theoretical Limit
All Etiologies of Disease
Application in NAFLD
DSI is Not Influenced by Steatosis in HCV Patients

**Graph 1:**
- Disease Severity Index (DSI) vs. Ishak Fibrosis
- $r^2 = 0.40$
- N=24

**Graph 2:**
- Disease Severity Index (DSI) vs. Biopsy Fat Score
- $r^2 = 0.02$
NASH Patient Demographics

31 Patients included in NASH pilot data set

- Biopsy Diagnosis:
  - 27 NASH
  - 4 Cryptogenic Cirrhosis
    (concurrent obesity, presumed NASH)
- Age: 54 ± 11 (mean ± SD), 26 – 71 (range)
- Gender: 65% male
- Brunt/Kleiner Fibrosis Stage
  - 4 at F1
  - 4 at F2
  - 5 at F3
  - 18 at F4 (cirrhosis)
- D’Amico/Zipprich Cirrhosis Stage
  - 4 at C1 (compensated, no varices)
  - 5 at C2 (compensated, with varices)
  - 4 at C3 (decompensated, no ascites)
  - 5 at C4 (decompensated, with ascites)
HepQuant® Function Map

- Portal HFR (mL/min/kg)
- Systemic HFR (mL/min/kg)

SHUNT: 100% 80% 60% 40%

DSI = 0
DSI = 20
DSI = 30
DSI = 40

DSI = 50

- SHUNT: 100% 80% 60% 40%
- DSI = 0
- DSI = 20
- DSI = 30
- DSI = 40

Graph showing the relationship between Portal HFR and Systemic HFR with different SHUNT and DSI values.
Liver Function in Controls and NASH Patients

<table>
<thead>
<tr>
<th></th>
<th>Portal HFR (mL/min/kg)</th>
<th>Systemic HFR (mL/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (BMI 18.5-25; n = 30)</td>
<td>102</td>
<td>51</td>
</tr>
<tr>
<td>Controls (BMI &gt; 25; n = 20)</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>NASH Fibrosis (Brunt 1-3; n = 13)</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>NASH Cirrhosis Comp (n = 9)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>NASH Cirrhosis Decomp (n = 9)</td>
<td>20%</td>
<td>10%</td>
</tr>
</tbody>
</table>

SHUNT: 100% 80% 60% 40%

DSI = 0 20%
DSI = 50 10%
DSI = 28
DSI = 16
DSI = 50

Controls (BMI 18.5-25; n = 30)
Controls (BMI > 25; n = 20)
NASH Fibrosis (Brunt 1-3; n = 13)
NASH Cirrhosis Comp (n = 9)
NASH Cirrhosis Decomp (n = 9)
Medium/Large Varices in NASH Patients

SHUNT: 100% 80% 60% 40%

Controls (BMI 18.5-25; n = 30)
Controls (BMI > 25; n = 20)
NASH w/o Med/Lg Varices (n = 22)
NASH w Med/Lg Varices (n = 9)
DSI Outperforms Brunt/Kleiner Fibrosis Stage in Identifying NASH Patients with Decompensation or with Medium/Large Varices

### Identifying NASH Patients with Decompensation

<table>
<thead>
<tr>
<th></th>
<th>ROC c-statistic</th>
<th>optimum cut-off</th>
<th>Sens.</th>
<th>Spec.</th>
<th>PPV</th>
<th>NPV</th>
<th>Youden Index (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HepQuant-SHUNT</td>
<td>0.98</td>
<td>DSI &gt; 28</td>
<td>100%</td>
<td>95%</td>
<td>90%</td>
<td>100%</td>
<td>0.95</td>
</tr>
<tr>
<td>Brunt/Kleiner</td>
<td>0.80</td>
<td>Cirrhosis</td>
<td>100%</td>
<td>59%</td>
<td>50%</td>
<td>100%</td>
<td>0.59</td>
</tr>
</tbody>
</table>

### Identifying NASH Patients with Medium/Large Varices

<table>
<thead>
<tr>
<th></th>
<th>ROC c-statistic</th>
<th>optimum cut-off</th>
<th>Sens.</th>
<th>Spec.</th>
<th>PPV</th>
<th>NPV</th>
<th>Youden Index (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HepQuant-SHUNT</td>
<td>0.92</td>
<td>DSI &gt; 28</td>
<td>89%</td>
<td>91%</td>
<td>80%</td>
<td>95%</td>
<td>0.80</td>
</tr>
<tr>
<td>Brunt/Kleiner</td>
<td>0.80</td>
<td>Cirrhosis</td>
<td>100%</td>
<td>59%</td>
<td>50%</td>
<td>100%</td>
<td>0.59</td>
</tr>
</tbody>
</table>
Measuring Treatment Effect
Measuring Change in DSI
Measuring Change in DSI

Δ DSI v3.3

- SOLAR 1 On-Rx Wk 4
- HALT C

p <0.002  p <0.02  p = NS  p <0.001  p = NS
## Using ΔDSI to Select Sample Size

<table>
<thead>
<tr>
<th></th>
<th>Mean ΔDSI Placebo group</th>
<th>Mean ΔDSI Treatment group</th>
<th>SD of ΔDSI</th>
<th>N per Arm 80% Power</th>
<th>N per Arm 90% Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>34</td>
<td>44</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>13</td>
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</tbody>
</table>
Use in Clinical Trials
HepQuant STAT

• Oral dose only – D4 cholate 40 mg
• One blood draw at 60 minutes
• Serum separated and shipped to HQ lab
• STAT, estimated DSI, reported
STAT Correlates with Fibrosis and Predicts Risk of Outcomes

- **Healthy Controls**: 30 patients (6 per stage)
- **HCV Patients**: 299 patients (6 to 58 per stage)
- **Correlations**: $R = 0.64$

### Clinical Outcomes

- **Proposed Cohorts for the Study**
  - Higher Risk for Outcomes: 58%
  - Lower Risk for Outcomes: 25%
  - $<0.80$: 6%

### Outcomes

- **CTP +2**: 18
- **Var Bleed**: 4
- **Ascites**: 8
- **Enceph**: 3
- **Asc+Enc**: 3
- **Death**: 16

### Ishak Fibrosis Stage Distribution

<table>
<thead>
<tr>
<th>Stage</th>
<th>Healthy Controls</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>220 HCV Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>299 HCV Patients</td>
<td>6</td>
<td>42</td>
<td>85</td>
<td>57</td>
<td>51</td>
<td>58</td>
<td>168</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Single (µM)</th>
<th>Healthy Controls</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>220 HCV Patients</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td>6</td>
<td>42</td>
<td>85</td>
<td>57</td>
<td>51</td>
<td>58</td>
<td>168</td>
</tr>
<tr>
<td>Test</td>
<td>Patient Procedures</td>
<td>Results</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT</td>
<td>Oral only, 1 draw</td>
<td>Estimates DSI</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>SHUNT</td>
<td>IV and Oral dosing, 5 draws</td>
<td>Portal Hepatic Filtration Rate (HFR), Systemic HFR, Portal-Systemic Shunt, DSI</td>
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**STAT** to Select; **SHUNT** to Monitor

**STAT**
- Screening a Population

**SHUNT**
- Monitoring Efficacy
- Selecting Patients by Disease Severity
- Interval DSI Assessments
Concluding Remarks

• Clinical outcomes are determined by the severity of functional impairment
• Early stages of disease may best be defined by functional tests
• Functional tests measure the state of liver disease in “real time”
• Functional tests can measure treatment effects – dose/response, time/action