Thank you very much. First of all, I thank John Senior for inviting me and Paul, of course. I have no financial disclosures. I will disclose that, as Paul said, I am a clinician. So, I am not well versed in the ways of the FDA or industry but I am learning a lot. If I say something stupid about your field of interest, please step up to the mike and publicly humiliate me in front of my peers. I will try not to take it personally.

Paul and John asked me to talk about this and it was really an exciting question and I realized there are absolutely no data in this area. So, that is good and bad. It means that the background is bad and there is not much to say in terms of background but I will do the best I can.
Outline

- Brief overview
  - Hy’s Law and Its Derivations
  - Hy’s Law Track Record (FDA and Registry data)
- Chronic liver disease patient outcomes and the DILIN experience
- Hy’s Law and chronic liver disease in the DILIN

This is the outline. I will be talking about Hy's Law and backtracking just a little bit, with a few slides about making sure we all have it right and we know what we are talking about in regard to Hy's Law and its derivations. I'll talk a little bit about the track record, which has been alluded to here quite a bit in the past two days.

And then quickly go into sort of chronic liver disease outcomes in relations to Hy's Law and in the DILIN experience. And then lastly, let us look at the new data that we just started putting together in the last several months. It is very preliminary but it will be getting right at the question that I have been asked to address: Hy's Law in chronic liver disease within DILIN.
First of all, I thought it was probably appropriate to go back to the man himself, in his last addition of his textbook. And this is what he said: "Drug-induced hepatocellular jaundice is a serious entity. The mortality rate ranges from 10% to 50%." We have seen that a lot. On the facing page, there is actually a table that I slimmed it down quite a bit, but he did put parameters on the enzymes. The AST and ALT were 3 to 50 times the upper limit of normal for hepatocellular injury, and he did put parameters on the alk phos, which was less than one to three times the upper limit of normal. You notice that he did not put any parameters on jaundice. It was a clinical call there.
Hy’s Law according to the FDA

• ALT or AST > 3 x ULN
• Bilirubin > 2 x ULN
• No “initial findings of cholestasis (elevated serum ALP)”
• No other reason for liver biochemistry elevations


This is Hy's Law according to the FDA and this is lifted straight from their guidance for industry.
So the AST and ALT are again, greater than three times, bilirubin there they did put a hard stop parameter of two times the upper limit of normal but they did not with alk phos.
Hy’s Law and Drug Trials

- ALT or AST > 3 x ULN—good sensitivity; poor specificity
- Bilirubin > 2x ULN – improves specificity
- “2 case rule”
  - Two HL cases found in a trial is felt to be highly predictive of the agent causing acute liver failure.

Basically they just say initial findings of cholestasis elevated serum alk phosphase and no further guidance there. And then there is obviously no reason for other liver biochemistries to get at causality here.
I would say a "highly predictive risk of acute liver failure", not DILI
Paul Watkins, 3/2/2015
Hy’s Law: Other Derivations

- US DILI Network (DILIN)*
  - ALT > 3x ULN
  - Bilirubin > 2x ULN
  - ALP < 2x ULN

- Spanish DILI Registry^:
  - ALT > 3x ULN
  - Bilirubin > 2x ULN
  - “excluding other (cholestatic) causes”
    or
  - \( \text{ALT or AST} \times \text{ULN/ALP} \times \text{ULN} > 5 \)
  - Bilirubin > 2x ULN

* Fontana, RF et al. Gastroenterology, 2014 (The US DILIN)

So, these are Hy’s Law’s other derivations. This is the top one which is our DILIN group and this is what we have used when we looked at this. Again, the ALT and bilirubin look very familiar. We do put a hard stop at alk phos less than two times the upper limit of normal. I also put up the Spanish and South American DILI Registry. They used a little bit different in two things. This is their most recent paper which I am sure some of the authors are out there and this was published last year. ALT and bilirubin are, again, the same. But they either used excluding other cholestatic causes but then they also used a new derivation which is incorporating the R-value. And here what they did was they took the AST or ALT, whichever was higher, and they put it times the upper limit of normal divided by the alk phos times the upper limit of normal and it had to be greater than five. And so they make the argument the alk phos sets a stand alone could probably be done away with and if you could just use the R-value. And their performance, at least in their study was better. Their RC curves were better for this, as opposed to a straightforward Hy’s Law.
So what about the track record in drug trials? This was shown yesterday quite a bit. I won't go into it much. This is bromfenac, troglitazone, and ximelagatran. So, these are sort of triumphs of Hy's Law that seem to pan out for post-marketing for the first two and then, obviously, the first one was not approved but later withdrawn from other markets.

### Track Record in Drug Trials:
Retrospective analyses & post-marketing

<table>
<thead>
<tr>
<th>Drug</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bromfenac</strong></td>
<td>• 2 HL cases in 1,195 in longer term use trial</td>
</tr>
<tr>
<td></td>
<td>• Predicted a 1:5,000 to 1:10,000 rate of severe DILI</td>
</tr>
<tr>
<td></td>
<td>• Post-marketing estimate: 1:10,000</td>
</tr>
<tr>
<td><strong>Troglitazone</strong></td>
<td>• 2 HL cases in 2,510 subjects in trial.</td>
</tr>
<tr>
<td></td>
<td>• Risk estimated at 1:10,000</td>
</tr>
<tr>
<td><strong>Ximelagatran</strong></td>
<td>• ~2 HL cases in 1000 subjects seen in longer term use trials</td>
</tr>
<tr>
<td></td>
<td>• Not approved in the US and later withdrawn in other countries</td>
</tr>
</tbody>
</table>

2. Lewis JH. Pharmacoeqi Drug Saf 2006
What about in registries? Hy's Law does very well in all the registries, really. This is the DILIN experience, 13.4 percent if you met Hy's Law in a hepatocellular injury, you had a 13.4 percent positive predicted value that you were not going to do well. That was mortality as an outcome.

Now the Spanish/South American Registry is very similar. Again, I told you they use two different models. They used a straightforward Hy's Law but also this modified one where they used the R-value. And there again it is between 8 and 9.6 percent. I do want to point out one nuance here. You know in the DILIN we use mortality. But if you read the paper carefully in the second one, they use mortality but they also use acute liver injury. In other words, bad synthetic dysfunction. And I will come back to that. I think that is important. You know, what are we defining as a bad outcome? It is a little different between the two.

And then the Swedish Adverse Drug Reactions, Dr. Bjornsson's registry out there. It shows you the number. This is how Hy's Law is panning out. It is somewhere around 10 percent, give or take a couple percentage points.
A word about chronic liver disease in DILI. Again, going back to what Dr. Zimmerman said, it is remarkable how much what he said did pan out over time. He said that there as a stubborn misconception that susceptibility was higher in patients with chronic liver disease. And he also said that addition to DILI to chronic liver disease would be troublesome. I get the feeling that is the general feeling across the field.

There are some data to support it. For example, the statin data suggest no increase in susceptibility, but on the other hand, there are some data that suggests that maybe it is a problem, for example in TB. When you monitor for TB, it is different. If you have chronic liver disease, you are monitoring ALTs. Or if you don’t you are just going on symptoms.

Before I go into some of the newer data that we have in relation to Hy’s Law in our chronic liver disease patients, it is good to review what we do in DILIN and what comes out adjudication. Basically, three of our members get together and independently score these cases and then come to consensus. This is the scoring system. I just want to go over it real quickly again. One is definite, greater than 95 percent likelihood beyond reasonable doubt that this is DILI. Two, highly likely, 75 to 95 percent, and probable 50 to 74 percent, based on the legal language in a court of law. So, basically, what we would say is that three or better would be enough to convict here. I highlight those because the rest of this data, just keep in mind, will be only dealing with cases that met those scores.
I will just talk a little bit about some backdrop data. This is the idiosyncratic DILIN experience within the first six months, morbidity and mortality. And I just want to give you an idea. We do have a measurable rate of bad outcomes. And these were 660 DILI cases, a six-month follow-up. We have the survival curves based on three different groups. Basically, liver transplant is the worst group, the solid black line. And we did a fair number fairly early on. Liver-related death is the next line. And then non-liver-related death is the line that lingers out a little bit longer. And I suspect those are a lot of the cancer patients.
Within this study, there are some hints that Hy's Law is still a player or helpful. We didn't break it out in this paper, as I will in a minute. But basically, preexisting liver disease was more common in those who had a death or transplant outcome. As you can see, 24 percent versus 11 percent. And if you restricted it just to liver-related death or transplant, again, it was statistically significantly higher for those with preexisting liver disease. Again, making Dr. Zimmerman's comment that it would be troublesome seem to be somewhat true here.

Now, Hy's Law was also more common in those with death or transplant outcome. Again, 46 to 26 percent and if you just restricted it to liver-related death or transplant, it was 53 to 26 percent, both statistically significant.

Now, when we looked at it as a multivariate model, both chronic liver disease and Hy's Law fell out of the multivariate model but I have to say there is a lot of collinearity here. Because you can see for Hy's Law, for example, ALT and bilirubin both stayed in the model. And for chronic liver disease, low platelets and low albumin both stayed in the model. So, I suspect if you took them out, then Hy's Law would slip back in and so would chronic liver disease.
Predicting Fatal Outcome
Hy’s Law in Chronic Liver Disease in the U.S. DILIN

- Cohort: n = 894 cases enrolled in DILIN
  - Only Definite, Highly likely and Probable cases.
- Subgroup analysis
  - Chronic liver disease (CLD) patients
    - HCV/HBV
    - NAFLD/Unexplained elevated liver biochemistries
  - Non-CLD patients
- Hy’s Law
  - ALT > 3 x ULN
  - Bilirubin > 2 x ULN
  - ALP < 2 x ULN

Okay, so this is the preliminary data that we have predicting fatal outcome in Hy’s Law. This is a cohort of now 894 patients, again, all definite, highly likely, or probable. And what I did was we looked at two groups, obviously, those with chronic liver disease going into the DILI and those without chronic liver disease. I later will subgroup them as viral hepatitis and, as best as we can tell, NAFLD and unexplained elevated liver biochemistries.
Predicting Fatal Outcome
Hy’s Law in Chronic Liver Disease in the U.S. DILIN

- Outcomes
  - DILIN Severity Index Score = 4 or 5
  - DILIN Severity Index Score = 5 (Death or Transplant)
- Death or Transplant
  - All cause, any time during follow-up.
  - Liver related death within 6 months or transplant within 6 months.

So, in the outcomes, this is where I come back to this. Now, in this analysis, I did do it both ways. Four is just actually you start to show liver failure; you develop ascites, encephalopathy, but you make it; you don’t get transplanted. You survive it. Five, of course, is death or transplant. I did a model on both but I am only going to show you the five data.
These are deaths or transplant. And I did it two different ways. All-cause, any time during follow-up. So, 1: you get transplanted anytime or die for no reason. And then 2: liver-related death within six months of transplant within six month.

**DILIN Severity Index Score**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mild</td>
<td>Patient has elevations in serum aminotransferase or alkaline phosphatase levels but total bilirubin is &lt; 2.5 mg/dL and there is no coagulopathy (INR &lt; 1.5).</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>Patient has elevations in serum aminotransferase or alkaline phosphatase levels and total bilirubin is ≥ 2.5 mg/dL or there is coagulopathy (INR ≥ 1.5) without hyperbilirubinemia.</td>
</tr>
<tr>
<td>3</td>
<td>Moderate-Hospitalized</td>
<td>Patient has elevations in serum aminotransferase or alkaline phosphatase levels and total bilirubin is ≥ 2.5 mg/dL or INR ≥ 1.5 and the patient is hospitalized (or a pre-existing hospitalization is prolonged) because of the drug-induced liver injury.</td>
</tr>
</tbody>
</table>
| 4     | Severe                       | Patient has elevations in serum aminotransferase or alkaline phosphatase levels and total bilirubin is ≥ 2.5 mg/dL and there is at least one of the following:  
  - signs of hepatic decompensation (INR ≥ 1.5, ascites, or encephalopathy), or  
  - other organ failure believed to be associated with drug-induced liver injury event. |
| 5     | Fatal                        | Patient dies or undergoes liver transplantation for drug-induced liver injury |
Demographic, clinical characteristics: it is a busy slide. But I will just highlight the fact that there really was no difference between a non-fatal, fatal, and total, except for age. As you might expect, the fatal group was a little bit older.

And then as far as chronic liver disease, individually, there was no real statistical difference. Even Hy's Law did not necessarily meet statistical significance, when we looked at liver-related within six months or liver transplant within six months.
If I expand it a little bit to follow-up at any time, death or liver transplant at any time, then Hy's Law does come back and is statistically significantly higher. So, again, if you got transplanted in month seven, I don't know, then that may be clinically significant or maybe that should be in there as a predictor for Hy's Law.

Okay, what I am going to show you next is a series of slides. They are all going to show the same thing as the tables. And I left the tables and numbers in because I think it is important for you know our numbers. They are not huge. They are bigger than whatever is out there. But as you can see, the numbers will whittle down as I go down and the outcomes change a little bit.
Cohort: probable, highly likely, definite cases

- **Outcome:**
  - *All-cause mortality, anytime during follow-up or*
  - *Liver transplant, anytime during follow-up*

- **Positive predictive value of Hy’s Law: 11%**

<table>
<thead>
<tr>
<th></th>
<th>Fatal Outcome</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>HysLaw</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>(+)</td>
<td>27</td>
<td>219</td>
</tr>
<tr>
<td>(-)</td>
<td>31</td>
<td>552</td>
</tr>
<tr>
<td>Totals</td>
<td>58</td>
<td>771</td>
</tr>
</tbody>
</table>

This is total cohort all-cause mortality. So, again, you could die for any reason, liver transplant at anytime during follow-up. So, just overall, again, comes out to about where Hy’s Law would say, about 11 percent, the positive predicted value. And you can look at the numbers there in the two-by-two.
I think some will be confused by this recurrent title when some slides don't list patients with chronic liver disease - I would just drop "and Chronic Liver Disease" from the titles and move "total Cohort and "No chronic Liver Disease" and "chronic liver disease" up to the title

Paul Watkins, 3/2/2015
No Chronic Liver Disease Patients

- **Outcome:**
  - *All-cause mortality, anytime during follow-up or*
  - *Liver transplant, anytime during follow-up*
  - **Positive predictive value of Hy’s Law: 9.5%**

<table>
<thead>
<tr>
<th>HysLaw</th>
<th>Fatal Outcome</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td>21</td>
<td>200</td>
</tr>
<tr>
<td>(-)</td>
<td>26</td>
<td>503</td>
</tr>
<tr>
<td>Totals</td>
<td>47</td>
<td>703</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>44.7</td>
</tr>
<tr>
<td>Specificity</td>
<td>71.6</td>
</tr>
<tr>
<td>PPV</td>
<td>9.5</td>
</tr>
<tr>
<td>NPV</td>
<td>95.1</td>
</tr>
<tr>
<td>Overall % w/ Fatal Outcome</td>
<td>6.3</td>
</tr>
</tbody>
</table>

So what about no chronic liver disease? Here about pretty close, similar. Again, all-cause mortality, liver transplant anytime and this is 9.5 percent positive predicted value.
When we restricted it to chronic liver disease patients, this is where we took a big jump. So, this would suggest that Hy's Law is of some worth. Again, all-cause mortality, anytime during follow-up or liver transplant, anytime during follow-up. This was a positive predicted value of 24 percent. Of course, the numbers are smaller. We had a total, in this analysis, we had 79 that had preexisting chronic liver disease. But again, the positive predicted value of the total 25 was 24 percent.
Then I put this as a final summary slide. Again, I wanted to show you the numbers but this is a summary of the last three slides I just showed you. All cohort, all-cause mortality, liver transplant at any time, 11 percent. But then for chronic and non-chronic liver disease, it was 9 percent versus 24 percent for chronic liver disease.

### Predicting Fatal Outcome
**Hy’s Law and Chronic Liver Disease**

- **Outcome:**
  - All-cause mortality, anytime during follow-up or
  - Liver transplant, anytime during follow-up

<table>
<thead>
<tr>
<th></th>
<th>Hy’s Law PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cohort</td>
<td>11.0%</td>
</tr>
<tr>
<td>No Chronic Liver Disease</td>
<td>9.5%</td>
</tr>
<tr>
<td>Chronic Liver Disease</td>
<td>24.0%</td>
</tr>
</tbody>
</table>
Cohort: probable, highly likely, definite cases

- **Outcome:**
  - Liver related death within 6 months and/or
  - Liver transplant within 6 months
- **Positive predictive value of Hy’s Law: 6.1%**

<table>
<thead>
<tr>
<th></th>
<th>Fatal Outcome</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>HysLaw (+)</td>
<td>15</td>
<td>246</td>
</tr>
<tr>
<td>HysLaw (-)</td>
<td>23</td>
<td>583</td>
</tr>
<tr>
<td>Totals</td>
<td>38</td>
<td>829</td>
</tr>
</tbody>
</table>

So, what about total cohort and liver-related death? So this is, again, this is a little different. We are restricting it on the other end of the scale. We are going to say that it is a death within six months that we feel is liver-related or a liver transplant within six months. And here the positive predicted value goes down a little bit. As I said, you might transplant somebody or have somebody die at six or eight months; they won't be in this outcome.

So, here 6.1 percent -- the numbers are pretty big because this is a total cohort -- positive predicted value.
And here it is for no chronic liver disease. We had no fatty-liver, as we know. We had no viral hepatitis. Again, a liver-related outcome within -- bad liver-related outcome within six months. Positive predicted value, again, is down to 5 percent.
And then, of course, what about chronic liver disease patients? Well, it stayed up there. Again, the numbers are small or smaller, I should say. But again, the number was still up to 16 percent for a short-term bad liver-related outcome.
Predicting Fatal Outcome
Hy’s Law and Chronic Liver Disease

- Outcome:
  - Liver related death within 6 months and/or
  - Liver transplant within 6 months

<table>
<thead>
<tr>
<th></th>
<th>Hy’s Law PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cohort</td>
<td>6.1%</td>
</tr>
<tr>
<td>No Chronic Liver Disease</td>
<td>5.0%</td>
</tr>
<tr>
<td>Chronic Liver Disease</td>
<td>16.0%</td>
</tr>
</tbody>
</table>

So, again, summarizing that. This is, again, a liver-related outcome in a short-term interval. Bad outcome is 6.1 percent for the total, 5 percent for the non-chronic liver disease, and 16 percent for the chronic liver disease.
Patients with Chronic HCV &/or HBV Infections

- Outcome:
  - *Liver related death within 6 months and/or*
  - *Liver transplant within 6 months*
  - **Positive predictive value of Hy’s Law: 15.4%**

<table>
<thead>
<tr>
<th></th>
<th>Fatal Outcome</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>HysLaw (+)</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>HysLaw (-)</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>Totals</td>
<td>3</td>
<td>36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>66.7</td>
</tr>
<tr>
<td>Specificity</td>
<td>66.7</td>
</tr>
<tr>
<td><strong>PPV</strong></td>
<td><strong>15.4</strong></td>
</tr>
<tr>
<td>NPV</td>
<td>95.7</td>
</tr>
<tr>
<td>Overall % w/ Fatal Outcome</td>
<td>8.3</td>
</tr>
</tbody>
</table>

So, a lot of people would be interested in what about viral hep versus fatty-liver. I did break it out for, again, the six-month outcomes. And it was 15.4 percent positive predicted value for patients with either hep C or hep B.
And then NAFLD or unexplained elevated liver enzymes, the positive predicted value is 8.3 percent.
Predicting Fatal Outcome
Hy’s Law and Chronic Liver Disease

• Outcome:
  • *Liver related death within 6 months and/or*
  • *Liver transplant within 6 months*

<table>
<thead>
<tr>
<th></th>
<th>Hy’s Law PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Liver Disease</td>
<td>16.0%</td>
</tr>
<tr>
<td>HCV and/or HBV</td>
<td>15.4%</td>
</tr>
<tr>
<td>NAFLD/unexplained elevated liver enzymes</td>
<td>8.3%</td>
</tr>
</tbody>
</table>

This is the summary, again, for those two groups. As you can see for the biohepatitis group, it is a little bit higher. Well, a fair amount higher but the numbers are even small.
Summary

- Patients with a fatal outcome in the U.S. DILIN tended to have more baseline chronic liver disease and have more cases fitting Hy’s Law.
- For those with chronic liver disease:
  - Hy’s Law had a positive predictive value (PPV) of 24% for all-cause, anytime fatality or transplant.
  - Hy’s Law had a positive predictive value (PPV) of 16% for liver related fatality or transplant within 6 months.
- Both of these PPVs were higher compared to those without chronic liver disease.
- The PPV for viral hepatitis patients may be higher than for NAFLD patients.

In summary, patients with a fatal outcome in the U.S. DILIN cohort tended to have more baseline chronic liver disease and have more cases fitting Hy's Law. That is in Bob Fontana’s paper that came out last year.

So, with those with chronic liver disease, so it is Hy's Law has a positive predicted value of 24 percent for all-cause anytime fatality or transplant. Hy's Law had a positive predicted value of 16 percent for liver-related deaths or transplant within six months. And both of these positive predicted values were higher compared to those without chronic liver disease.

The positive predicted value for viral hepatitis patients may be higher than that, but I caution you that the numbers will get pretty darn small there.
The conclusions from this very preliminary data say that Hy’s Law may have a predictive value for fatality or transplant in patients with chronic liver disease than in those without. Whether or how this translates into overall incidence and risk for acute liver failure in a drug trial using chronic liver disease subjects is unclear, but suggests a continuing role for Hy’s Law.

Further research should focus on validation of these findings in other cohorts and adjusting Hy’s Law parameters for best accuracy in those with chronic liver disease.

The conclusions from this very preliminary data say that Hy's Law may have a predictive value for fatality or transplant patients with chronic liver disease than those without. Whether or how this translates into overall incidence and risk for acute liver failure in a drug trial using chronic liver disease subjects is unclear, but suggests a continuing role for Hy’s Law.

Further research should focus on validations of these findings in other cohorts and maybe adjusting Hy’s Laws parameters. Because if it is even more predictive, then maybe the parameters need to be dialed in a little differently. The caveats here, this is preliminary data. We were just looking at this data. I have not looked. For example, there is hep B. What does that mean? Were they hep B carriers? Were they active. We have not broken that data out. The hep C, were they treated? Probably not. Most of these were the pre-oral agent era. But again, we haven't broken all that out.

And the last thing is this death causality. I will mention that. I think we are looking at some cases and it is another parameter. I have heard a lot about how we have to set standards up but how do you tribute the death to the drug, when you have a liver go down? So, I will give you an example. For example, we have had a case of DRESS. The patient died but at the time of death, the liver was sort of on the mend. Now, is that a liver-related death or not? Things like that are a little more nuanced and we are taking that on to look at it that more closely. And that may change what I have shown here for positive predicted values but I don’t think greatly.
I want to thank everybody from the DILIN group, and especially Sherry Gu, who is in the upper right-hand corner of the picture. She is our statistician who put all this together for me today. Thank you.