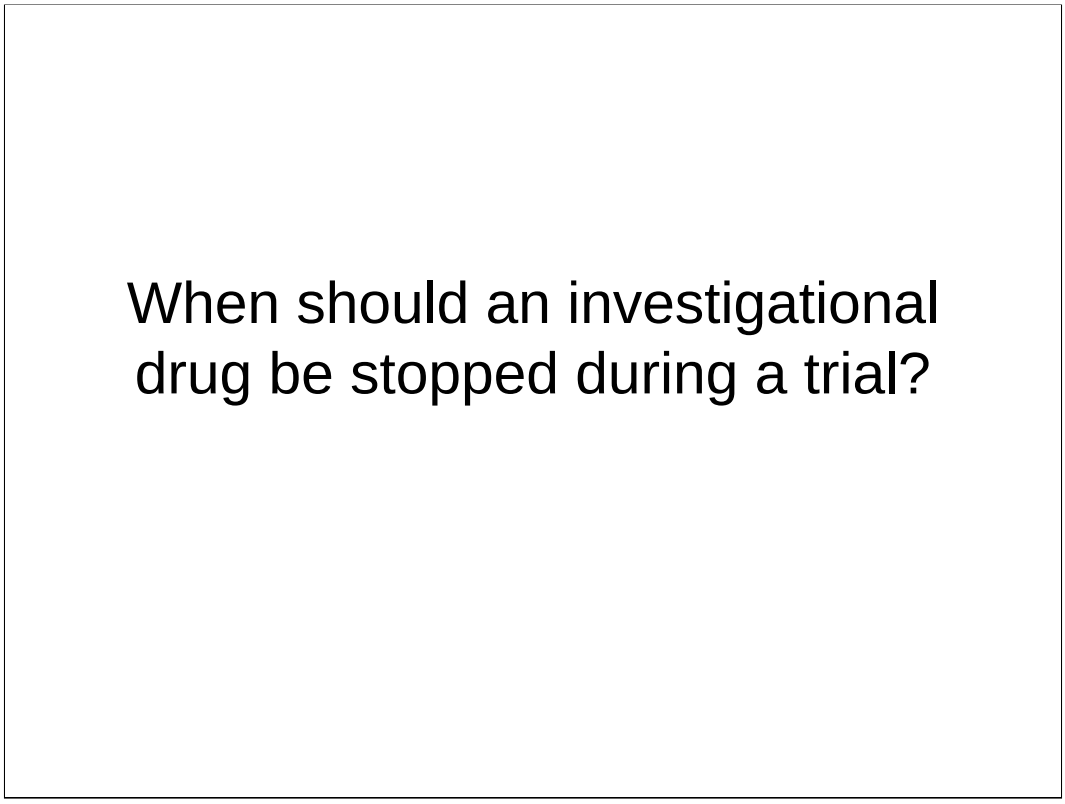


DR. WATKINS: Okay. So when do you stop treatment in a clinical trial? In the draft guidance it says when the ALT or AST goes greater than eight times the upper limit of normal. We already heard about issues on the upper limit of normal. We heard from Jack Bloom that Lilly, apparently sometimes, uses their “extended upper limit of normal” which would be 120 U/L. Therefore, we would be talking about an ALT of 1,000 or almost so at 960 U/L. So I think that points out this is something that we need to put some more thought into in terms of writing guidance. The point was made that the Guidance doesn't say late in development and doesn't distinguish what phase of the drug development we're talking about. I think we would assume this would be late in development, Phase III clinical trials, when it might be reasonable to go up to eight times the upper limit of normal in an asymptomatic person. And that's the way the Guidance should probably read.

Liver chemistries

Stop if:

- ALT or AST > 8 X ULN
- ALT or AST > 5 X ULN for more than 2 weeks
- ALT or AST > 3 X ULN and TBL > 2 X ULN or INR > 1.5.
- ALT or AST > 3X ULN with *the appearance of worsening* fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia.

AST or ALT greater than 5 times for more than 2 weeks should also be considered, because the guideline says if it's over 3 times, bring the patient back within 48-72 hours and if they continue to be higher than 5 times but less than 8, to stop the drug. And then ALT or AST greater than 2 times ULN, with total bilirubin greater than 2 times, that's a Hy's Law case, but we might also consider an INR greater than 1.5.

For ALT or AST greater than three times the upper limit of normal, with the appearance of worsening fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia, may also be reasons for stopping the experimental drug. That implies that you don't necessarily have to stop just because the ALT is greater than three times, and the subject has symptoms suggestive of liver disease, because it says the appearance of worsening fatigue, but maybe that's something we can ask for some clarification on. Next.

Monitoring Frequency

In general, in early studies of a drug in study subjects with presumably normal liver function should involve obtaining liver tests every 2-4 weeks, for at least a few months…. In longer trials, if there is no sign of liver injury after a reasonable length of exposure (eg. 3 months), the monitoring frequency can be increased to every 2-3 months.

Hy's Law was covered in the first session. To go with the three components of Hy's observation: 1) drug-caused hepatocellular injury, generally shown by more frequent threefold or greater elevations of upper limit of normal of ALT or AST than the nonhepatotoxic control agent or placebo. That's the Temple's Corollary. Next. Among subjects showing such ALT elevations, the document actually says "aminotransferase" and makes no distinctions between ALT and AST. (2) There are subjects who also show elevation of serum total bilirubin greater than two times ULN without initial findings of cholestatis. We asked for the basis for the two times upper limit of normal; there are no data to support that, but it seemed reasonable, and we didn't hear any objections.

And finally, (3) we thought we should add no reason can be found to explain the combination of the increased aminotransferases and total bilirubin, such as viral hepatitis A, B or C, preexisting or acute liver disease, or another drug capable of causing liver injury. We discussed the fact that conjugated bilirubin isn't mentioned, and the answer was the FDA simply doesn't get a lot of data on conjugated bilirubin because routine protocols don't require its determination. There may also be some issues in terms of the accuracy of direct-reacting bilirubin to measure of conjugated bilirubin.

Symptoms

If symptoms compatible with DILI precede knowledge of serum abnormalities, liver enzyme measurements should be made immediately, regardless of the next visit or monitoring interval is scheduled….

Attention to symptoms does not supplant routine periodic assessment of AT, TBL, and ALP…

And then ALT/AST greater than three times the upper limit of normal with the appearance of worsening fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia. That implies that you don't necessarily have to stop just because the ALT is greater than three times, and the subject has symptoms suggestive of liver disease because it says the appearance of worsening fatigue, but maybe that's something we can ask for some clarification on.

Hy's Law cases have three components

1). The drug causes hepatocellular injury, generally shown by more frequent 3-fold or greater elevations above ULN of ALT or AST than the (nonhepatotoxic) control agent or placebo

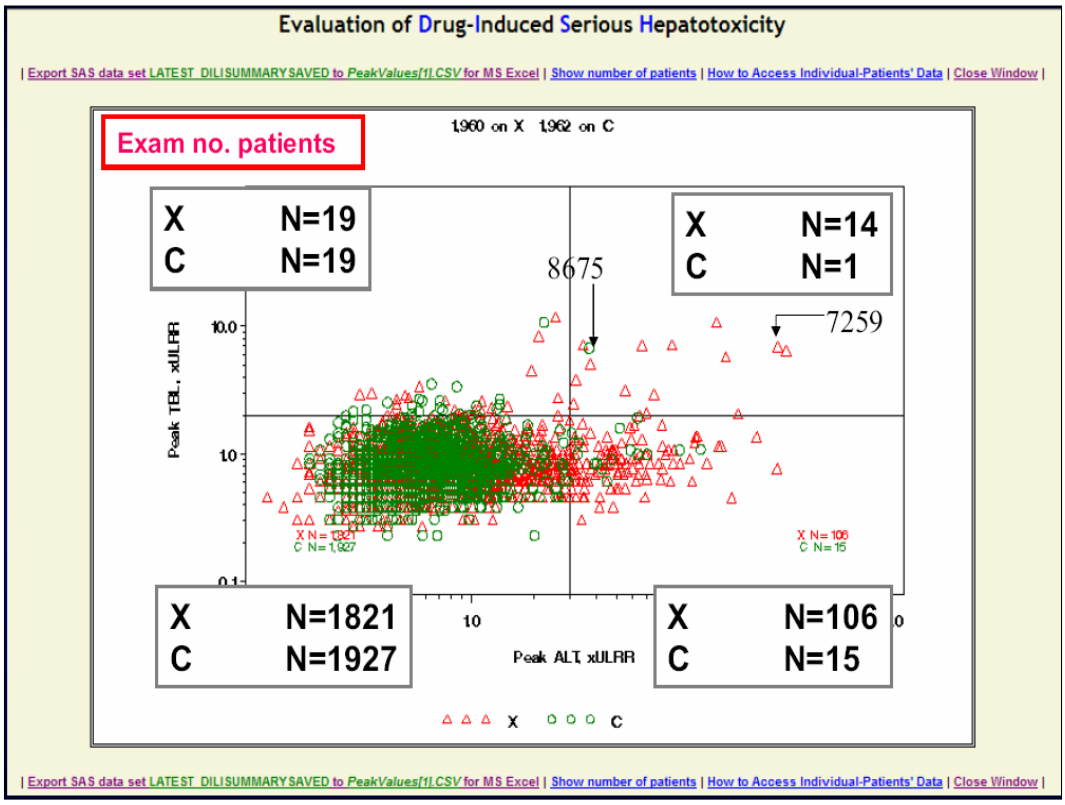
2). Among subjects showing such AT elevations, often with ATs much greater than 3 X ULN, some subjects also show elevation of serum TBL to > 2 X ULN, without initial findings of cholestasis (serum alkaline phosphatase (ALP) activity > 2 X ULN).

Hy's Law was covered in the first session. To go with the three components of Hy's observation: 1) drug-caused hepatocellular injury, generally shown by more frequent threefold or greater elevations of upper limit of normal of ALT or AST than the nonhepatotoxic control agent or placebo. That's the Temple's Corollary. Next. Among subjects showing such ALT elevations, the document actually says "aminotransferase" and makes no distinctions between ALT and AST. (2) There are subjects who also show elevation of serum total bilirubin greater than two times ULN without initial findings of cholestatis. We asked for the basis for the two times upper limit of normal; there are no data to support that, but it seemed reasonable, and we didn't hear any objections.

Hy's Law cases have three components

3). No other *(more likely)* reason can be found to explain the combination of increased AT and TBL, such as viral hepatitis A, B, or C, preexisting or acute liver disease, or another drug capable of causing the observed injury.

And finally, (3) we thought we should add no reason can be found to explain the combination of the increased aminotransferases and total bilirubin, such as viral hepatitis A, B or C, preexisting or acute liver disease, or another drug capable of causing liver injury. We discussed the fact that conjugated bilirubin isn't mentioned, and the answer was the FDA simply doesn't get a lot of data on conjugated bilirubin because routine protocols don't require its determination. There may also be some issues in terms of the accuracy of direct-reacting bilirubin to measure of conjugated bilirubin.



My final slide, in summary, is really a presentation we had on eDISH. This is a similar idea as is commonly used in the genomics data realm; visualization of complex data sets by graphing the maximum ALT along the X axis and the maximum total bilirubin along the Y axis. By adding lines corresponding to 3 X ULN for ALT and 2 X ULN for bilirubin, you develop the four quadrants. The two of interest for regulators and for you all are the ones on the right. So the lower right-hand is Temple's Corollary where the ALT is greater than three times but the bilirubin is not elevated. And then the upper right-hand corner are potential Hy's Law cases. By having different symbols for control and treated subjects, you get a lot of information from this display. I mean it might be you could get a panel of experts that would go into every one of those triangles in the upper right-hand corner and come up with an alternate explanation, but the obvious question is then why aren't there any green triangles (controls treatment cases, or only one, in that right upper quadrant. The eDish disply shows you this immediately.

And then the whole idea of the first step in Hy's Law is that these potential Hy's Law cases emerge from a subset of people that have lower level of injury, the lower right-hand quadrant. One possibility is that the Guidance should actually state that if there're any liver safety issues, the data have to be either presented in eDish form or to make it very easy for the regulators to do this sort of thing. And, in fact, the eDISH program now allows you to go to one triangle with your mouse, click on it, and actually get the patient number and go right in and directly retrieve all relevant subject data.

This display would be helpful well beyond regulatory approval issues. For instance, in the study that John has proposed with isoniazid, if this were the drug isoniazid illustrated here, there are many people in the right lower quadrant, but with continued treatment, most of them go back to the left lower quadrant, but a few go onto the right upper quadrant. Obviously if you have saved serum and/or urine for metabolomics, etc., on these people, you could begin to ask what's different about those that adapt versus those that don't adapt.

Now what Frank Sistare said was another issue, which is that some drugs do this like troglitazone have treated patients in both the right lower quadrant and the right upper quadrant but there are other drugs like tacrine that only have people in the right lower quadrant and apparently never have anybody who goes to the right upper quadrant. If biological samples had been saved from troglitazone clinical trials and from tacrine clinical trails, you could go and say, well, what is the difference? Let's find biomarkers that distinguish a benign drug that gives and ALT signal only versus a drug that causes ALT and bili elevations.

And a comment that's always made is that industry has a very short memory. The FDA is inundated with piles of boxes which they burn sooner or later (laughter). There's no way to go back and easily retrieve this information. And it's over simplifying slightly but if the next 100 NDAs that get approved use eDish and created this graph, you could literally flip through the graphs like a cartoon and pick the drugs that you wanted to go back to retrieve the stored serum or genomic DNA. So it would be a huge, huge advantage to have liver safety data presented in this way. So anyway, on that note, I will stop and I think, John, you wanted to have questions?