100 percent positive agreement and 99.6 percent negative agreement and can be run from a colony sweep with 100 percent positive agreement and 100 percent negative agreement, agreement with EIA. The test was also compared to CTA and performed at 100 percent when compared to CTA.

It is, to tie it back to something that Dr. Tarr mentioned this morning when one of the panelists asked what would be your ideal test. Before he said molecular his first comment was ideally while the patient is still in the emergency room. We have the only available test to be able to do that.

There is another recent cleared test on the market but it requires an incubation period first. So, with this test I think you may all find some ability to get the versatility of what EIA is able to do for you but yet with a much shorter time frame. There is no need to batch and run this test. It can be run one at a time. Or, if they wanted to set it up and run 10 or 15 at once, they could do it that way as well.

The test format is optical immunoassay. It is a flat silicon chip that is streaked with antibodies and, when you place the stool sample on this chip, there is a 10-minute incubation period followed by adding a drop of substrate and then there is a 5-minute incubation period.

So there is minimal handling. There is no centrifugation, no mixing, no vortexing. The test design, itself, is a permanent record. It will last for years and years and years and you guys can hold on to those. We have had customers keep those for years, not with this test but with other disease states.

It is not prone to clogging or flow issues as lateral flow tests normally are. Most lateral flow tests, as we all know them, would be, say, your standard pregnancy test or things like that where there is wicking action that draws the test up. In this case, because there is no migration of the sample, it provides an extremely optimal format for looking for this disease state, for toxins.

So I understand what Dr. Tarr was

mentioning before regarding use of toxin as opposed to a different way but, in comparison to EIA, I think you would all find that it provides a nice format for you.

Any questions? I would be happy to answer them.

DR. RELLER: Thank you, Mr. Stern. There being no other speakers in the Open Public Session, we will proceed directly to our next presentation entitled STEC Disease Severity Score, by Dr. Martin Bitzan from McGill University.

STEC Disease Severity Score

DR. BITZAN: Good afternoon, advisory committee and FDA members. Thank you very much for inviting me to present you the data that I would like to present.

[Slide]

I would like to make a disclosure statement. I am an unpaid clinical consultant for Thallion, formerly Caprion, primarily motivated by my work and the clinical experience with children with hemolytic uremic syndrome. I was also the

recipient of a modest unrestricted research grant from Caprion.

[Slide]

The objectives of this presentation are first to discuss the concept of Shiga toxin-mediated events or, in short, STME, and to propose and discuss with you a scoring system for the severity of infections by Shiga toxin-producing bacteria, or STEC.

[Slide]

For the past 20 years our community was concerned with the treatment and prevention of HUS, but I think, as we discussed this morning, it is time to think of an alternative approach to STEC disease. This approach would be different from the HUS-centered perspective and should be possibly able to better encompass and judge the effects that Shiga toxin by itself has on the organism, on the patient.

In order to identify items that may be relevant for the disease expression of Shiga toxin I set out to identify a set of Shiga toxin-related

clinical and laboratory changes--and they will be termed in the future of this talk Shiga toxin-mediated events or STMEB-under the assumption that reducing Shiga toxin-mediated events, including HUS, would be a proof of principle for any intervention that might be contemplated in this disease and, on the other hand, under the assumption that if such a reduction can be shown, other Shiga toxin-related complications, if they are being reduced, would also predict that HUS by itself will be reduced.

[Slide]

How do we define those events? It is relatively clear. These are signs and symptoms that are directly or indirectly attributable to the biological action of Shiga toxins based on animal models, and on clinical pathological observations. And, we have heard a great deal of that already this morning.

[Slide]

In this table I listed several key features of Shiga toxin-mediated disease and I used

only animal models that are produced by injection models in order to have the purest form of disease that is really attributable to the toxin and not to other bacterial factors. It is obvious from these models that watery diarrhea and bloody diarrhea can be produced in rabbits and in rats which have been intravenously injected with the toxin. There are animal models that we have seen which have other aspects of human HUS, although none of these models is perfect. And, we already assume and know that hemolysis, and thrombocytopenia and renal injury is related to Shiga toxin.

In order to drive that point home, that hemorrhagic colitis by itself is a toxin-mediated disease, I would like to explain this slide to you which originates from an injected rabbit with Shiga toxin 1 in this case, and sacrificed after two hours, where one then detected the toxin by indirect immunofluorescence in the small feeding mucosal vessels of the colon and of the cecum of this animal. These are exactly the sites that then later on, when we observe the animal for another

two or three days, show a tremendous amount of edema and of hemorrhagic infiltrates and of thrombotic microangiopathy, exactly the feature that we are targeting when we are looking at HUS.

[Slide]

So, in order to develop the system we asked what would be the ideal features for a scoring system that would then allow us to grade the extent of Shiga toxin-mediated events. The system should be simple; should be qualitative. It should be containing clinical symptoms that are easy to assess by nonprofessionals. It should show concordance with observers and it should have a combination of clinical and laboratory parameters, and it should be related to important clinical outcomes.

[Slide]

Based on clinical experience and literature review, a set of candidate clinical laboratory signs was assembled, Shiga toxin-mediated events, and the grading system adapted from the Common Terminology Criteria for

Adverse Events, probably familiar to most in the audience, which is very commonly used in clinical investigations and many investigators are familiar with this grading system of adverse events. We have adapted that to a situation that reflects Shiga toxin-producing E. coli disease. Then I went about to evaluate in a retrospective analysis a large cohort of children in our hospital, Montreal Children=s Hospital, who had bona fide Shiga toxin E. coli 0157 infections.

[Slide]

This disease scale that I am going to explain contains five major categories. Four categories are usually found, enteropathy, vasculopathy and coagulation, the signs of microhemopathic, hemolytic anemia and nephropathy, and in rare patients, unfortunately, extra intestinal and extra renal complication.

[Slide]

Here is an example of the enteropathy part of this proposed scale. On the left-hand column you see the essential components of hemorrhagic

colitis of frequent diarrhea, abdominal pain and cramps and bloody diarrhea. On the X axis you see the grading system directly adapted from the CTCAE scale, ranging from 0-4 and grade 5 would be reserved for death according to their criteria.

[Slide]

The next slide shows a few other criteria of the scale component that are essential for this disease. They are more prominently found in patients with HUS or with extra intestinal complications and include hemolytic anemia, thrombocytopenia, hematuria as a sign of kidney disease and elevated serum creatinine as a rough, imperfect but frequently used parameter of kidney dysfunction.

[Slide]

So, here is a little overview on the database just so you understand where the data have been derived from. I was able to identify, with the help of our microbiology records, 186 patients with identified E. coli 0157 infection over a period of 14 years. One hundred and sixty-four

patients had clinical records available that could be evaluated, and the population captured in this group is six years old and ranges from three months to 18 years. The majority, as we expect, had any type of diarrhea; 84 percent and 16 percent in this cohort had HUS.

Now, there is a referral bias because there were 12 patients involved who had been transferred from other hospitals to our institution. If I subtract those I would arrive at a number of eight percent, which is probably more realistic for many centers in North America.

It needs to be pointed out that 33 percent of the total cohort had been hospitalized and six percent dialyzed, about 40 percent of the HUS patients, and one patient had died.

[Slide]

In this first table what I would like to do is to address the question whether the scores that have been incorporated in the scale are, indeed, clinically relevant. So, with the help of my collaborator and statistician, Dr. Bill

Blackwelder, did an analysis, univariate logistic regression and, not surprisingly, in the lower part you will see-Band I will just briefly explain the scale again. On the left part you have the criteria, the scale items, and the number of patients who had data to contribute, and not every patient had all the lab results drawn. What you see, not surprisingly, is that, indeed, hemoglobin, platelet number and dysfunction of the kidney was highly significantly associated for patients with HUS. So, this is good. Then we looked at the other patients. To our surprise, diarrhea frequency actually came up as significantly associated with HUS.

[Slide]

In the next step of the analysis I looked at the correlation between Shiga toxin-mediated events and the scoring of the patients with these events and the duration of hospitalization. Again not surprisingly, there is quite good correlation between the parameters that define HUS, anemia, thrombocytopenia and renal dysfunction, but there

is also significant correlation between the eneteropathy parameters.

[Slide]

In order to clarify the picture a little bit we excluded the patients with HUS and just looked at the patients who had diarrhea, and looked whether there the same correlation would exist with duration of hospitalization. Almost the same numbers show up here in terms of p values and correlation coefficients according to sperm [ph] and rank correlation that we had seen in the previous table, which gives me a lot of comfort that these are, number one, clinically relevant parameters we measure and that they are not biased or significantly biased by the appearance of HUS. So, we defined parameters which are, by themselves, associated with Shiga toxin-caused morbidity.

[Slide]

We then went a step further and asked would these parameters actually contribute to hospitalization by itself and to what extent. It is interesting to see that any one of those

parameters, at least by univariate analysis, shows significantly increasing amount of hospitalization with each step of the scoring system. So, the higher the score, that translates directly into higher estimated risk of hospitalization.

[Slide]

Now, what you see here is the depiction of the course of a patient of not so difficult HUS. This patient had HUS but was not dialyzed, so falls into an easier category according to Dr. Tarr. Actually, you see in the lower part the various clinical parameters as they evolved, the type of diarrhea and the laboratory parameters. But it would take me probably 15 minutes to walk you through the whole slide. So, let=s skip that and go to the next one.

[Slide]

What I show you here is actually the graphic depiction of the scoring system. So, on the X axis you see the days since onset of the disease and on the You axis the scores for each of these parameters that are listed on the right side.

For example, the disease starts with watery diarrhea, in white; then abdominal cramps appear; and then-I can=t see it exactly-on day three there is bloody diarrhea. And, you see that this is a biphasic disease. So, this is the enteropathy phase. Between day six and day seven, boom, something happens here. On the same day, with the same measurements, thrombocytopenia appears, hematuria appears, hemolytic anemia appears and the creatinine starts to rise above the normalized threshold for that age group. Then you see the evolution of the disease. This is just a compilation of the scores, and the scores are designed to be simple, linear, one point for each degree of severity.

[Slide]

What you see here is a comparison between several types of Shiga toxin-producing E. coli infections, and they all scored, again on day-to-day scoring and compilation of those scores on the You axis. This is actually the same patient we just discussed. Then there is a patient with

so-called incomplete HUS with minor features of the HUS, and a patient who does not meet any criteria of HUS and has only watery non-bloody diarrhea, bloody diarrhea and abdominal cramps, which gives us a visual idea of how severe or less severe the Shiga toxin-mediated disease can present.

[Slide]

In conclusion, I would like to summarize that Shiga toxin-mediated events, as defined here, are measurable biological effects of Shiga toxin; that the Shiga toxin disease severity scale items are associated with clinically relevant outcomes; and that this system allows to integrate disease severity and duration; and that this could be an interesting tool for standardized documentation and evaluation of STEC disease among patient groups, among different strains of bacteria, among different areas and geographical regions; and could also be useful for prospective studies, be it preventive studies or therapeutic interventions. That is the end of my talk. Thank you very much. I will be happy to answer questions.

DR. RELLER: Questions for Dr. Bitzan? Yes, Dr. Cnaan?

DR. CNAAN: I have a clarification question. When you say in your early slides on the various factors maximal score, that means over the duration of the hospitalization?

DR. BITZAN: Correct. I apologize that this did not become clear. If we just look at this graph, you see that this patient had an increasing scoring over the days for various symptoms and for each of these symptoms. For example, if we take bloody diarrhea onB-I think it is day 4, 5 and 6, this patient has a maximal score of 3 for the bloody diarrhea. So, this would be incorporated into that table. If we take creatinine so I think it is a 2 or 3 score for creatinine, for renal dysfunction, that would then be incorporated into that table.

DR. RELLER: Dr. Tarr?

DR. TARR: How do you take into account absent points? It looks like on this particular patient you have no laboratory values prior to day

7 and all of a sudden the colors change when you get laboratory values.

DR. BITZAN: Okay, that is true. You do not usually have data until the patient arrives at the hospital. So, in this case there was lab achieved on day 5. They were all in the normal range. I think I can go back one more. So, what you see in this conventional depiction is that hemoglobin was normal. The platelets were normal. I apologize, these are international units. And the creatinine was entirely normal at that point. So, the next data point is already deranged and we ideally would have an intermediate value, which we do not have.

DR. TARR: And can you relate the kinetics? In other words, a platelet count of 200,000 on Wednesday whereas it had been 400,000 the day before is quite concerning in this setting but it is still normal. Do you have day-to-day relationships in this model?

DR. BITZAN: It is an excellent question because in the patient who progresses to develop

HUS this is a significant finding. A patient who has a certain degree of dehydration or something else, or a virus disease that just preceded may not be something else important and does not meet the diagnostic criteria definition of HUS. Correct. In the initial attempt to capture that, I would have given that degree of 1 but for the sake of clarity I did not because suddenly you would be confused by patients who have plain diarrhea and never develop HUS. So, yes, that is another concern.

DR. TARR: Finally, by bringing in all these additional variables, do you run the risk of making a difficult to power study even more difficult by adding in more outcomes rather than the simple HUS yes/no, severe HUS yes/no?

DR. BITZAN: I am not going into the details, but I think it actually opens the opportunity to have an idea of the severity of the disease burden rather than the opposite. So, assumingB-the assumption certainly has to be proven that the chosen items, and the scale is imperfect

at the moment, but one can certainly prove that by using other parameters, validating them better. I think that actually gives us a better idea of how severe the disease is than just the definition of mild or severe. And if you want to then compare and you can weight the curves, then that might be a way to go.

DR. RELLER: Dr. Hilton and then Kaskel and Ward.

DR. HILTON: Could we look at the slide again with the three cases side by side? To me, it looks like the three measures at the bottom could be omitted from the score because they are almost the same in all three cases.

DR. BITZAN: Almost. I didn=t do the full calculation. They are different, some of them. If that is so and they are not being affected by treatment, then they can certainly be omitted. But I hope if there is any effective intervention, whatever this may be and that would affect the severity of the diarrhea and the crampiness of the abdominal pain, then actually this curve would

change and there would be more rapid evolution of these signs. So, then you would possibly be able to pick up differences. But as I pointed out, 85, 90 percent of the patients will never develop this colorful part here and they will stay with the brown part.

DR. HILTON: My last point is just that the other components, the laboratory components maybe overlap quite strongly and give pretty much the same information.

DR. BITZAN: You are right in the sense if somebody has mild or severe HUS you are probably fine without the enteropathy part. But my point was the enteropathy by itself is a Shiga toxin-mediated disease expression. Now, provided one could intervene early enough I would predict that there is a benefit to be achieved. I struggled to find a way how I can express that and this is one attempt.

DR. RELLER: Dr. Kaskel?

DR. KASKEL: Some of these patients present with hypertension or develop it shortly after

admission, and for long-term outcome, even if the creatinine comes back to normal in a patient who required dialysis, what we are left with is patients at risk for progression of renal disease if they, indeed, have hypertension or persistent proteinuria and I think we have to be looking at some data as to what happens with these outcome measures early on.

DR. BITZAN: I cannot agree with you more as a nephrologist. But, again, this is a minority of patients, a very small group, who are very important because they are the ones who may develop need for transplantation. If one has a desire to grade them in any way or to categorize them in a predictive analysis, which is not the case here, one can focus on this group. But I think the majority of patients who have an infection by Shiga toxin-producing bacteria will not develop that severe sequelae and I would like to know whether I can learn and compare patients who have the milder form of disease that is an expression of Shiga toxin effect andB-that is the whole of this

discussion--which by itself could be a reflection of the efficacy, or the strategy that then can be extrapolated to efficacy for HUS prevention in the light of having so few patients who really have HUS.

DR. RELLER: Dr. Ward?

DR. WARD: This is what I was trying to get to this morning, that some sort of a compound or a combination of clinical symptoms and laboratory data scoring system I think is very helpful at helping to encompass the spectrum of the disease. I suspect though that there are actually available data through either the CDC or through Dr. Tarr=s patient group, or maybe yours in Montreal, that would allow you to do a multiple logistic regression that would predict on what you think are the most predictive values, and you can then go back to another set and prospectively test it now without having to collect new patients.

DR. BITZAN: This is a very interesting and important step to do, but for the sake of this discussion we are looking at the severity of the

disease in order to compare different circumstances where the disease becomes severe. If the question is who is going to develop HUS, which is very relevant for patients and the parents, and so forth, and for the hospital and for the society as such, this is important. Unfortunately, any retrospective analysis is plagued by missing data and by incomplete data sets. If I would do a study today I would ask Dr. Tarr and see all we have are these and these parameters that we really want to include, and not only the routine parameters. I had to deal with routine parameters that have been done in many laboratories.

DR. RELLER: Dr. Wong-Beringer?

DR. WONG-BERINGER: I guess I just want to follow-up on my prior question. I know this is looking at the retrospective, existing data that you have. I wonder if you can comment on the role and feasibility of adding a parameter that measures genetic predisposition.

MR. BITZAN: I think it is still the Holy Grail of many people to find risk factors not only

epidemiologically defined but genetically defined. I think we are still in infancy because Dr. Tarr had raised several important attempts, logical attempts and they did not result in clear answers of genetic risk factors. And, in order to pick up the question from this morning about Gb3, there was a lot of enthusiasm for a while to come to some profile and I, myself, had tried to correlate blood group P antigens which are Gb3 and Gb4 and Gb5 related substances which by itself bind in vitro very well toxin. It even binds red blood cells if they are treated in a certain way. But it doesn=t seem to be the way how it acts in the body.

No, so far even the differences in the lipid side chain that have been studied extensively by Dr. Lingwood in Toronto, and his group, did not allow and they did not really even attempt to study that in a larger group of population, allowed to pinpoint stable differences. You have under certain circumstances changes in the membrane and lipid barrier. You will have changes in that, according to nutrition, even in how the composition

is. But it is not sufficient to dissect or predict risk, at least not in one particular species, as far as I understand the studies and discussed it with him.

There is also controversy about how and whether, for example, young children have more Gb3 expressed in the kidney cortex and endothelial cells of the cortical vessels than adults and whether that would be a true risk factor. This has been actually disputed. There are other studies showing that kidney biopsy samples from all age groups have more or less the same amount of Gb3. So, there must be something in addition that is important. But, on the other hand, you cannot expect any toxin effect without any Gb3.

DR. WONG-BERINGER: Thank you.

DR. RELLER: Dr. Rappley?

DR. RAPPLEY: Can you describe how treatment might have changed over the 14 years that your patient population was evolving? And, in your institution there, in Montreal, do you use aggressive hydration, and what would you

characterize as close to a standard of care that we might compare a new product with?

DR. BITZAN: Thank you for that question. Unfortunately, treatment has not changed very much over the 14 years. What has changed is the rapidity to identify children that are possibly at risk. So, today they just come earlier, as you have pointed out, Dr. Tarr, because the population is much more aware. This probably has somewhat of a positive impact, although the studies from Dr. Ziegler don=t seem to show that as a huge difference.

The treatment itselfB-again, we are talking about one or two patients with true HUS in our emergency room population per year, or three maybe. It depends on the year. Certainly, the nephrology community takes note of Dr. Tarr=s paper and it makes sense to put the emphasis on proper hydration because it makes absolute sense if the patient is dehydrated that there is multiplying of risk. I cannot say that we have officially instituted a particular regime of saline

infusion-Bwe are talking about isotonic saline, not necessarily hydrationB-in order to achieve that. But I think nobody can ignore those data and not do it. But I am not really aware of a standard of care, but I think everybody knows today that we have to give fluids rather than stop giving fluids, which was done for a certain time period. We are more relaxed in immediately jumping on dialysis because we know that some patients do resolve in a day or two. And, we are trying to do as much as we can through supportive treatment, including parenteral nutrition if that is necessary and pain medication to alleviate the sometimes horrendous abdominal pain. By the way, the abdominal pain is often the leading factor subjectively to go to the hospital and to be admitted to a hospital.

DR. RELLER: Dr. Tarr?

DR. TARR: Over your 14-year analysis have there been any trends in frequency? Is it going down? Up? About stable?

DR. BITZAN: More up and down. I didn=t calculate it out for the years. It is certainly on

my mind to do. There were in the early years more patients than in the final years that I have subjectively seen, and we are seeing certainlyB-I have the impression particularly over the last three or four years that there are less patients than are in the databaseB-I am only two years in Montreal nowB-from the earlier years. In the early >90s there were more patients than there are now but I can=t give you exact distribution over the years.

DR. RELLER: Doctor?

DR. WIEDERMANN: Thank you. This is a little bit related to I think what Dr. Ward was talking about. I am having a tough time understanding your scoring system. I understand the odds ratios and the correlation coefficients. Then, like a lot of scoring systems, you have changed continuous and interval variables into ordinal variables and you come up with a score. And, I don=t hear anything about how that was validated or how it could be used, and I am having a disconnect here for what this scoring system

means.

DR. BITZAN: To clarify the order of evolution, the scoring system was perceived based on literature reports and my own clinical experience, not on statistical data. The next step was to apply them to the retrospective data set. I have only recently begun to more rigorously analyze them, together with my collaborator, and this is not yet final. There is probably more we can do.

In the idealized situation one would then come up and say have a more rigorous statistical system to support that. But I don=t think that the goal was to have something that could predict too much or toB-it is hard to formulate it for me-Bthe evolution is exactly the opposite way. Now, you could now go ahead and say, well, I would like to establish those and those criteria. I take that as an example and then I really build the model according to that. I am only beginning to propose something that I find potentially useful.

DR. WIEDERMANN: Right, but I think for a treatment study, which is what we are hoping for,

if you can say a score of 12 means that there is a 68 percent likelihood of progression to mild HUS or, you know, then you see if intervention alters that, you know, I think that is where we could be headed.

DR. BITZAN: I think the potential is there to do exactly that. I will go back to the drawing board and try to calculate that out. So far I looked at this more as a relational base, which means I just compare intervention plus/minus and see whether there is a difference, rather than finding a predictor at 12 points that will progress to HUS. That was not the initial intention.

DR. RELLER: Dr. Cnaan?

DR. CNAAN: I have two questions, one which was exactly that, is there a cutoff and that question is, therefore, foregone. My other question is do you view this measure as a surrogate for HUS, a precursor to HUS, or do you view it as simply another clinical outcome worthy of pursuit?

DR. BITZAN: Good, thank you. It is probably the third one of your explanations. It is

an instrument, a tool to put numbers on something that is clinically evident. It is not very sophisticated. The plan was to have something simple. Does that answer the question?

DR. CNAAN: I guess part of our focus today is to try to see what we can do to test HUS. I guess if I am looking at it totally it is different outcome. It is simple. It is easy to use. All of that is very nice. I am trying to get to can it serve in answering the HUS question or it answers a different question.

DR. BITZAN: Okay, I think I understood your question better. Thank you. The whole goal is to expand the idea of Shiga toxin-mediated disease and to include--for the sake of evaluation of an intervention, include more parameters than only HUS. So, it doesn=t help me to diagnose HUS or it may help a little bit to find another way to grade HUS, but that really requires rigorous statistics to substantiate that. No, it helps you more in the milder form Shiga toxin disease, I hope.

DR. RELLER: Dr. Gorman?

DR. GORMAN: Assuming your scale turns out to be validated in whatever method people choose to validate it, for an outcome measure for an intervention would you hope that we would see an interruption in the rise of the scale or a reduction in the highest point on the scale?

DR. BITZAN: There are several ways one could use such a scale and I think you addressed two of them. You could measure the changes, the dynamic of the change. You could measure the total burden. You could measure the difference between the total burden in a group. These are possibilities and I would have to rely on a statistician to tell me what the power calculations and the calculations were for this. I am not a statistician. I have to rely on my collaborators for this.

DR. GORMAN: Well, I am not a statistician either so I will let the statisticians do that. The question I would then ask is are there points on that scale that you would consider more

important to reduce than others? Because if an intervention interferes with the amount of cramping I am not sure most clinicians in this room would consider that the most important score to reduce. So, if you reduce the score of the cramps from 4 to 0, that would be an important one for the patient perhaps over the short term but not important in terms of the progression of this disease. So, I am asking you to consider weighting these as you go forward.

DR. BITZAN: Yes, thank you for this question. There will be, I am sure, many attempts to think about something and usually weighting goes into the considerations. The point here, again, is to find a tool not only to say this helps me to bring the justification for the treatment in the real world, but it is a tool to tell me, I hope, whether Shiga toxin disease can be influenced for a trial or for a comparison. It is not a justification. I agree with you. If somebody has watery diarrhea for 3 days and maybe a little bit of blood tinge and goes home, I am wondering

whether that by itself would justify an expensive treatment. But if you want to study the efficacy of an intervention it may broaden the base to compare efficacy by Shiga toxin disease.

DR. RELLER: To follow-up on Dr. Cnaan=s question, if one cannot make a connection with the antecedent gut events and some way to predict progression to mild or severe hemolytic uremic syndrome, isn=t one left with a self-limited GI infection to which known antimicrobials, given or not given, contribute little or nothing?

My point is that if you cannot make some connection between the enteropathy part of the score and the adverse outcome then it would seem to me conceivable that one might have interventions that would affect the gut but would have nothing to do with HUS. Hence, the importance I think of Dr. Cnaan=s trying to get to what is the use of this. Is that correct?

DR. BITZAN: I understand your question and I would like to reiterate two things. One is that whenever somebody does a trial and uses a scale

like this, if the HUS does not go into the same direction, the rate reduction of HUS, the severity of HUS is not ameliorated or prevented in the same direction as the other symptoms--hemorrhagic colitis, enteropathy parameters--then there is a disconnect and this is not at all a proof of efficacy for this score. So, the first assumption is that there is a relationship between the disease as it plays out in the gut. The mechanism is very similar and I think Dr. Tarr will agree with me. And, the disease plays out in other regions of the body. Why somebody develops the illness is a big question.

So, I assume, and that has to be studied, that there will be a similar effect. If that similar effect is not playing out, then the study is not valid in that sense. But I assume that there is a correlation and an association, and we saw, at least with the number I presented, that there is a certain agreement between the higher scores and the development of HUS.

Now, Dr. Tarr will probably tell me, or

some other people will tell me, well, we have so many people with hemorrhagic colitis, what does that tell me? And, we saw in one of the calculations that actually it did not even show up as a statistical variant because the vast majority has that sign. But if you look according to the severity, and there is a certain degree of training necessary to grade the severity properly of the hematochesia, of the blood in the stool, then there is a correlation. So, I am quite optimistic that these enteropathy symptoms reflect the same mechanism and have an association with the HUS.

DR. RELLER: Other questions for Dr. Bitzan? If not, thank you very much. Oh, Dr. Daum?

DR. DAUM: I am trying to grapple with the score, how I would interpret a change in the score effected by treatment X. It seems to me, and I think this is inherent to the process of a score, I don=t think it is any fault of your systemB-inherent to it is that anything worth 1 point or 2 points or 3 points has an equal weight

in predicting bad outcomes or bad course that we care about. My guess is that is probably not true with the available information that we have, with lots of indecisions and perhaps several factors sort of impinging on each other to give more weight than 1 plus 1 doesn=t make 2; it might make 3.

So, without knowing exactly which of the crucial elements of this you could use your system, have a wonderful reduction in score that is statistically significant, and everything else that people who know statistics would say and have no impact at all on the long-term outcome of the disease. So, I am worried that without knowing precisely how to weight the different elements you have that getting a conclusion of a reduction would be uninterpretable.

DR. BITZAN: This dilemma, as you point out, exists in many areas. As a nephrologist, I deal almost on a daily basis with the Banff classification scheme where one looks at parameters to diagnose rejection of a renal transplant, with a scoring system which is 1, 2, 3 usually, and has

several elements, and which refrains from having a really quantitative system. So, you go according to morphological criteria and assume that there is a correlation with the degree of rejection or the severity of rejection and do, over time, studies to validate that or at least come to agreement that they, indeed, predict or correlate with certain degrees. I think it is in the ballpark of these kind of scales and scores.

Excuse me, what was the second part of your question?

DR. DAUM: There really was only one part, and you may have addressed it. I guess the other thought that I had is that it is all about weighting, it seems to me. You made the point that the score goes up as the disease gets worse. Well, you also do more testing as the disease gets worse. If I understood the scoring system correctly, if you had an unknown creatinine you wouldn=t assign any points to that. It would only be if you actually did the test.

DR. BITZAN: You are absolutely right that

this needs daily or at least clear set interval measurements, and I tested that out in a cohort which actually had daily observations and, luckily, there was no HUS so I cannot really look at that point. What I do see though is that those patients who do develop evidence of thrombotic microangiopathy, their score actually goes significantly up, I wouldn=t say significantly but it goes very much higher. So, as soon as a patient develops those extra intestinal complications there are many more points added to that scale. So, I would not be concerned that we are missing out on important criteria because we don=t weight them.

DR. RELLER: Thank you very much. The time has arrived for an industry perspective. We will begin with Thallion Pharmaceuticals= presentation.

Thallion Pharmaceuticals

Trial Design for Shiga Toxin-Producing Bacterial Infection

DR. CLEARY: My name is Tom Cleary. I am a pediatric infectious disease specialist at the University of Texas Medical School in Houston. I

am at the Center for Infectious Disease in the School of Public Health and the Department of Pediatrics Medical School.

[Slide]

The only financial disclosure I have to report is that I am a consultant for Thallion and am presenting the plan that we have been working on to try to evaluate a monoclonal antibody strategy.

[Slide]

You already heard today that this is a disease that represents a cascade of events, a cascade that starts with intestinal colonization by STEC, followed by production and uptake of Shiga toxin 1 or Shiga toxin 2 or both toxins. In the setting where endothelial cell receptors are up-regulated by cytokines, there is binding to the neutral glycolipid Gb3, internalization of toxin, inhibition of protein synthesis and vascular endothelial injury.

[Slide]

That sequence of events, that cascade relates to a series of clinical events that are

described on this slide. After ingestion, a few days later, 1, 2, 3, varying by the number of organisms ingested presumably and on the child=s immunity, is development of diarrheal disease. That is followed in a few days by development of either grossly bloody diarrhea or blood-streaked diarrhea, so-called hemorrhagic colitis, typically with little or not fever. Coagulopathy, depending on how you define it, develops in 15, 30 or more percent of patients, depending on what parameters you use. Dr. Tarr talked this morning about changes in D-dimers and prothrombin fragment 1 pus 2, and so on. So, there is a whole series of abnormalities that are going on in coagulation fairly early in the disease. A subset of kids who have this vascular endothelial injury syndrome and the resulting coagulopathy go on and develop HUS. Some of these children die. Some of these children end up on dialysis for a long time. Some of these children end up with other long-term sequelae.

[Slide]

The challenge to a therapeutic

intervention is that there is no method to detect either of the circulating toxin in humans, presumably because the half-life is so short that the toxin is not there very long and, therefore, not typically detected. That makes it difficult to know when a monoclonal antibody strategy might be useful. Clearly, there is a point in time where giving monoclonals is likely to be not very helpful. A child already is anuric with HUS probably isn=t going to get better by a monoclonal antibody.

The second problem is that predicting who will develop HUS during STEC infection is impossible. Again, you heard earlier today from Dr. Tarr about the abnormalities that he sees in kids prior to development of HUS. But you also heard the overlap that exists between the kids who develop HUS and the kids who don=t develop HUS, and the inability to predict who will develop HUS in the individual child.

You have heard ad nauseam today about the rarity of the disease, the difficulty of studying,

the impossibility of using HUS as an outcome. We think that the way to do this really has to focus on timing as a critically important variable; that it is critical to have rapid diagnosis. It is critical to have early intervention. It is then critical to be able to somehow measure outcomes that include HUS but include other clinically relevant parameters.

[Slide]

We have been inspired by the work that Dr. Bitzan has done because it has really given us the first tool to try to quantitate disease. Is every child who has HUS equivalent? Well, obviously not if some of them go on and die and others don=t even need to be dialyzed. Is every child who has hemorrhagic colitis equivalent? Again, obviously not. The child who has a little blood-streaked diarrhea and doesn=t need to be hospitalized is very different from the child who develops severe, profuse bloody diarrhea and perhaps is in the hospital for a week or ten days.

So, there is this gradation of severity

that results and it is a gradation that, if we can quantitate, we may be able to show that we have blocked events that are clinically relevant. So, we anticipate that early interruption of Shiga toxin-mediated events will alleviate the rate and severity of illness as measured by the sort of toxin severity scale or disease severity scale that Dr. Bitzan has talked about.

[Slide]

In this way of thinking about things, clearly there is a critical window of opportunity for monoclonal antibodies to be helpful. We are not sure precisely what that window is. On this slide we have shown it as the first 3 days perhaps of illness. It may be that the line should be drawn at day 4 or day 5. We are not really sure. But it is likely that the toxin-mediated events, the events that Dr. Bitzan has talked about that are related specifically to toxin getting into blood. If we can do something about those events we can do something about the downstream cascade and that, we think, is really critical.

[Slide]

The monoclonal antibodies that we propose to use are antibodies directed against both toxin 1 and toxin 2. They are chimeric IgG1 monoclonals that in the subsequent parts of the slides are going to be referred to by this nomenclature, here. These bind exclusively, in the case of the antibody to toxin 1 to toxin 1 and, in the case of the antibody to toxin 2, binds exclusively to toxin 2. It should be pointed out that the majority of North American STEC infections are with organisms that encode both toxin 1 and toxin 2. We all have the impression that toxin 2 is the most important but it is also true that toxin 1 is being made by the majority of strains and is likely to have a role in disease. Clearly, for example, you can take baboons and give them toxin 1 only and they develop what looks, for all the world, like HUS.

[Slide]

Now, the antibody to toxin 1 targets the B subunit. It is an antibody that in the literature was originally called 13c4. It is an IgG1

antibody, described originally by Strockbine in 1985. It immunoprecipitates toxin 1. It blocks toxin 1 binding to Gb3. The epitope that it recognizes is 3 noncontiguous segments on the B subunit of toxin 1. It neutralizes toxin 1 in Vero cell assays and protects animals in a murine toxemia model.

[Slide]

The antibody to toxin 2 is to the A subunit. It is an antibody that in the literature was originally called 11e10. It is an IgG1 antibody. It immunoprecipitates toxin 2. The epitope recognizes the N-terminal region of the A subunit. It neutralizes the cytotoxicity of toxin 2, toxin 2c and toxin 2d activatable Vero cells. This is very important because, as you heard earlier today, the spinach outbreak, for example, was due to a strain that produces both toxin 2 and toxin 2c. These 3 variants that are mentioned here appear to be the major variants of toxin 2 that are important in terms of severe human disease such as hemolytic uremic syndrome.

An important proof of concept is that this antibody will rescue animals from dying when administered up to 48 or 72 hours post infection in a murine model. So, the mice are infected with a toxin 2 producing strain, you can give antibody even a couple of days afterward and protect the animals from dying.

[Slide]

Preclinical toxicology studies where the antibodies have been used alone and in combination showed that they are not associated with significant or serious toxicity in healthy animals, mice and marmosets, or in infected animals, mice. These two antibodies did not exacerbate the course of disease in the animals that survived. These two antibodies did not activate complement in a kidney-cell tissue culture model.

[Slide]

Dr. Ward had asked earlier for some data showing that these had safety in humans. There have been Phase I studies to show that in healthy adult volunteers, 50 volunteers, given these

antibodies alone or in combination the antibodies were safe and well tolerated, a total of four studies that have been done.

PK studies showed that the antibodies have a half-life of about nine days at the 3 mg/kg dose. Antibodies to these antibodies, human anti-chimeric antibody response occurs in the anticipated range based on other similar products.

[Slide]

This slide simply gives you an idea about what sorts of adverse events have occurred in these 50 patients over a range of doses, and they are similar to the sorts of adverse events that typically occur with similar products.

[Slide]

So, the rationale for a proposed randomized, controlled, double-blind Phase II/III study is, first of all, there is that an urgency to develop something that is an effective intervention. Obviously, the outbreak with spinach last fall underscores again that kids continue to die from a disease that because of the cascade, the

timing of events looks like we ought to be able to interrupt, we ought to be able to do something about it.

The second issue is that the rare disease truly fits under the definition of an orphan indication. It is critical to optimize the data collection, and that is why we are proposing this combined Phase II/III. Obviously, this is not going to be an easy study. The disease is unpredictable in its occurrence. There are going to be some significant recruitment issues. If we are only going to accept patients who have toxin in their stool and are early in disease, some of the patients will roll in at a point where they have had bloody diarrhea for three or four days and they are pretty late, and they may be just about to have HUS. They are not very good subjects to demonstrate whether or not such intervention is likely to be effective. So, with the sorts of numbers you heard earlier today the study is likely to be even more difficult than that. For that reason, it is anticipated that at least 50 sites

internationally will need to be involved in the study and that will yield an estimated one patient per site per month.

[Slide]

This is an overview of part A of this Phase II/III trial. The purpose of this part will be to determine the safety of this antibody combination in STEC infected children, and to determine what dose should be used in the subsequent part of the study. The doses tested here, the range is based on Phase I and animal model data. Basically ten times the amount of antibody needed to protect mice from dying is the amount that has been used.

In this scheme, the top part, the four boxes on the top, show randomization at the low dose, the 1 mg/kg dose. Fifteen children will get that dose. Ten others will be randomized to placebo. At day 14 of the last patient there will be a safety analysis to make sure that problems haven=t occurred in kids that we wouldn=t have predicted from the 50 adults that have already been

studied.

The second part, if no safety concerns evolve, will be to look at a higher dose. In this part, five placebo and 15 high dose administrations will be done. Again, after the last patient is 14 days from administration of the drug there will be a safety analysis before deciding to move on to the larger clinical study.

[Slide]

The larger clinical study looks something like this, 135 children will be randomized to placebo; 135 children will be randomized to the largest safety dose shown in part A. So, if the 3 mg/kg dose has the same frequency of headache and drowsiness and such as occurs with the lower dose, we will use the larger dose. So, there will be 135 in each of these groups. In addition, the 15 placebos from part A will be added to the placebo group here so there will be a total of 150 placebo. Also, the 15 at whichever ended up using from the part A will be added in so there will be 150 in that group. Obviously, the primary focus here is

going to be on efficacy, although safety measures, again, are going to be critically important.

[Slide]

The population that we propose studying is a population of kids starting from about six months of age, going up to 18 years. Obviously, in places like South America we expect the vast majority of disease to occur between six months and four years. In North America we expect more kids up to age ten, and there are these occasional kids who are a bit older. So, the kids who fit the right sort of clinical profile who are older than ten we are also willing to have.

Diarrhea for no more than three consecutive days is the way we are currently envisioning this, but we would certainly appreciate the wisdom of the committee in terms of whether or not we ought to perhaps accept kids at four or five days if they have bloody diarrhea perhaps for a day or less. It is critically important here for this rapid intervention strategy that the stools have to be looked at and we get a diagnosis very quickly

before proceeding so the direct stool assay you heard about after lunch, the Biostar Shigatox assay, a 15-minute assay that gives you results that can be acted on at the time you see the child in the emergency room, that is going to be the test that is going to be used to decide who gets placebo or antibody. This test has just very recently been approved, approved in January and became commercially available on February 9th. So, this is sort of fresh off the press and really represents a unique opportunity for us to really do early intervention studies and figure out whether or not this strategy is going to work or not.

[Slide]

Now, endpoint considerations. We anticipate that we will see a decrease in HUS. If we don=t, we got no drug. But for sample size reasons that you have heard about all day long, it is crystal-clear we are not going to be able, in any reasonable sized study, to show a statistically significant decrease in HUS. We expect less HUS and milder HUS but it is unlikely we will achieve

statistical significance. However, using the STME approach that Dr. Bitzan has talked about, we hope to be able to show a shift in the severity of the spectrum of disease, the less severe HUS to milder coagulopathy, milder enteropathy. I would like to illustrate that in just a couple of minutes.

[Slide]

So, the primary endpoint, we are anticipating, will be looked at in one of several ways. In the packet we have talked about the first of these two approaches, looking at the proportion of patients who have an STME progression, that is, developing a new STME or having an increase by two points on the Bitzan scale in STME.

We have also been considering a different approach that is not in your packet and that we are thinking may actually be a better way to go. It is a way that gets around some of the questions that you had for Dr. Bitzan because it becomes much more quantitative, much more description of the overall course of disease. That is, to look at total disease burden as indicated by daily cumulative

STME scores over the 14 days post dose. For sample size calculations, a 50 percent difference between groups in these measures was anticipated.

[Slide]

This just gives you an idea about what the two approaches might look like. So, first STME progression. At the bottom left you see a red arrow with no new STME. The idea here is we have a child who started with a normal serum creatinine and went to a creatinine above the upper limit of normal for age but not two times the upper limit. That is a change of 1 in the scale, going from normal to 1.

The other red arrow, up above, shows moving from an abnormal baseline to an increase in score by 2. Obviously, these scores have a certain arbitrariness to them. Obviously, they might deserve somewhat different weighting. We have argued about whether or not the thrombotic microangiopathy shouldn=t be scored 0, 1, 2, 3, 4 but 0,1, 3, 5, 7 9 because we think that is the most important outcome. But because Dr. Bitzan has

clearly shown that the severity of the diarrhea has a huge impact on hospitalization, we think that preventing hospitalization may be a big deal too and should be part of what we are looking at. So, that is the reason for the scale this way right now.

[Slide]

This is to give an idea of what total disease burden might look like. The little pink boxes here are meant to be representative of the findings on a given patient on a given day during his illness. For example, he might have a score of 1 for diarrhea, 2 for abdominal pain and bloody diarrhea, 3 for evidence of low hemoglobin and low platelets, 4 for having anuria and dialysis. Well, this child who is anuric, being dialyzed, has a low hemoglobin, has hemolytic anemia, has thrombocytopenia everybody recognizes this is a severe HUS. This is a child who may be in the hospital for 4 or 6 weeks on dialysis.

[Slide]

In contrast, this is also a child with

HUS. This child has a low hemoglobin, a low platelet count, has a little bit of hematuria and has an elevated creatinine. He has HUS.

We think that if we can, in our severity scale, show a shift in severity of disease from that first child to that second child=s score, that is potentially a big deal. Ending up with kids who aren=t on dialysis and don=t have those long hospitalizations may be a very, very useful outcome. We think this scale is going to be able to allow us to quantitate that kind of information.

[Slide]

This is just looking at what you might get in several representative patients for a cumulative score. We have added up the scores on a daily basis during 14 days so the red line at the top-Band this is a slide I borrowed from Dr. Bitzan for purposes of demonstrating--the red line at the top shows the child he had shown who developed HUS but didn=t need to be dialyzed. His cumulative score during the 14 days is 145.

The child with the sort of yellow line-BI

am not sure what color it looks like up there, on my screen it is yellow--is a child who has thrombocytopenia and hemolytic anemia but never developed any evidence of renal involvement, what is sometimes called incomplete HUS or form-first HUS. It is evidence of the coagulopathy but not ending up with a disaster. He has a cumulative score, you can see, that is significantly lower, 64.

The child who just has hemorrhagic colitis that is uncomplicated, on the other hand, has a cumulative score during the 14 days of 25.

So, having kids who still develop HUS but don=t end up with these high scores reflecting prolonged hospitalization and severe illness might be a very, very biologically relevant clinical outcome to be looking at.

[Slide]

So, in summary, evaluation in animals and in human volunteers suggests that the product is likely to be safe in children. You never know. You have to look at it in kids. You have to look

at it in kids st STEC disease. Maybe there is something unexpected. We will certainly be carefully watching for that. But the reasonable prediction at this point in time is this is likely to not be a dangerous intervention.

The proposed Phase II/III design for this orphan indication is for early intervention and we think early intervention is feasible with the sort of design we are anticipating.

Using the STME scale, we hope to demonstrate--we propose to demonstrate clinically relevant decreases in severity of disease. The example I gave you of the child who has sever HUS and is in the hospital for a month versus the child treated with antibody who ends up with mild HUS, at home in a week is a perfect example. An equally reasonable example is the child who comes in with watery diarrhea, progresses to severe hemorrhagic colitis, is hospitalized for a week or ten days versus the child who gets antibody, comes in with watery diarrhea and that is as bad as it gets. He is never even hospitalized.

So, we think that the STME scale provides potentially a very powerful tool to quantitate something that otherwise we treat as categorical, HUS/no HUS; hemorrhagic colitis; no hemorrhagic colitis. We think the ability to try to quantitate this is the way to get at do we have an effective or an ineffective intervention.

A major advantage of the proposed approach is that we are using the combination of antibodies, antibody to toxin 1, antibody to toxin 2, and we are doing it early in the course of the illness, at a time when we hope to block effectively the toxicity of both toxin 1 and toxin 2 mediated disease.

At this point let me stop. My colleagues from Thallion and I will be happy to try to answer any questions you might have.

DR. RELLER: Questions? Dr. Smith?

DR. M. SMITH: I have two questions. One, you were talking about using the assay for diarrhea stools but one of the things that we know about this disease is that you may not have the stool

present or there may be few stools. Is the sensitivity of the assay on a stool sample the same as, say, like Dr. Tarr had for doing a swab? That is the first question.

DR. CLEARY: Perhaps the person from Biostar could talk about that. Sensitivity on swab versus stool sampleB-
the data I know is on stool sample.

DR. M. SMITH: Right.

DR. CLEARY: Do you have any specific data on swabs?

THALLION: In the product insert the test was tested against EIA for direct stools; was tested for broth culture; and was tested for colony sweep. It was not tested via collection methods other than that. So, people could validate it off of the swab but I just have to talk about it as listed in the product insert.

DR. CLEARY: The important thing is the correlation between that assay as done with fecal samples. Presumably not many of these were with swabs. Between that assay and either cytotoxicity

assay or the Meridian EIA, considering the Meridian EIA the gold standard, you know, between 98 and 100 percent for both sensitivity and specificity so that the ability to pick up kids tonight when you see them in the emergency roomB-rather, when you see them tonight you set up a McConkey sorbitol, you set up an overnight broth, you eventually do the Meridian EIA and late tomorrow afternoon you decide, well, that would be a good one to have randomized yesterday. So, we think that is incredibly important for this kind of intervention aimed at going at the earliest possible time point.

DR. M. SMITH: Then, my next question is looking at the two arms of the study that you are proposing and knowing that Dr. Tarr has recently introduced this information about hydration, whether you want to call it weight-based or however, would it be feasible, considering this is a rare disease, adding a third arm where you have your second therapeutic option as some weight-based normal saline or something like that?

DR. CLEARY: We propose to follow the

standard of care as outlined by the IDSA. Okay? The IDSA standard of care says no antimotility agents, no antibiotics, hydration. They haven=t yet addressed the issue of the study that Dr. Tarr has put together. It is a very important study. I suspect it may well be right. The problem is it is clearly preliminary data. It is 29 patients. My bias is he is right but it is hard for me to impose that on the whole world based on my bias that he is right. So, I think that that is not going to be part of the protocol. I think it is going to be expected that the kids will be well hydrated, that appropriate hydration will be done, but the sort of large fluid boluses, the large saline boluses that we are talking about, we are hesitant to do that until there are more studies. We think it is a very, very important question but we don=t think it can be answered in this setting where we are already trying to answer a question about an outcome that is difficult enough to get at without throwing in a whole other variable. Phil, do you want to add anything to that?

DR. TARR: I understand what you are saying, but these are the only 29 patients who have ever been published.

DR. CLEARY: It is an ideal setting actually for a very carefully done animal study where you could get a crystal-clear answer without half the kids in the bad outcome group having gotten antibiotics for example, and so forth.

DR. TARR: I would like to point out that if you don=t have the most rigorous nephroprotective support for all patients in the study you will bias yourself towards finding an effect that might not be appropriate.

DR. CLEARY: I think there is no question that the standard of care is to have the kids appropriately hydrated.

DR. TARR: Yes.

DR. CLEARY: What precisely is the definition of appropriately hydrated kids I think we could debate for a long time obviously, and we don=t really know whether that means you ought to load them with sodium and water to the extent that

is suggested in the study because the study, admittedly, is very small. You know, it is an important question and it really needs to be answered in a stand-alone study.

DR. TARR: I would like to point out that we have never admitted a patient to our study or to my care on or before day 4 of illness who subsequently required dialysis. So, at least in one severe outcome there is a strong association with not meeting one of your case definitions. Good hydration is strongly associated in our experience. Even though this is only 29 patients--

DR. CLEARY: Yes, exactly.

DR. TARR: B-it was only a carve-out of a much larger study because we wanted to keep each of those 29 patients quite defensible. It was only the Children=s Hospital cohort. There are probably two, two and a half times as many patients overall that we didn=t publish. So, I think that I would be hard-pressed to say you are a child who has a risk of developing kidney failure in the next week. Half of that kidney failure is going to be acute

tubular necrosis; go home.

DR. CLEARY: Right. Well, as I said, I think everybody would agree on having the kids adequately hydrated that NTN does not occur.

DR. TARR: I would like to divert another question. Assuming that you can find enough sites and assuming you can enroll enough patients, the next key, the next critical point here is going to be this test. Can we get some more data on this test? What were the stools that went into the validation of the test?

DR. RELLER: Dr. Tarr asked this question and is Jonathan Stern available to answer that component?

DR. STERN: From what I understand, they were a mixture of semi-solid, liquid, watery and bloody stools, all types like that. As I say, it was tested on fresh stool, broth. It was done with frozen as well. It was done with all methods.

DR. CLEARY: Also, there is a subsequent study that is going to be published in AThe Journal of Clinical Micro@ by Park and O=Brien, where they

also had very similar outcome to what is reported in the package insert.

DR. TARR: let me follow up the question. Where were these stools collected?

DR. STERN: Well, there were different sites. There were different clinical trial sites but the main author on the abstract that was at ASM last year and V-Tech was done by Dr. Park here, at ANOVA, Fairfax.

DR. TARR: And, were they frozen stools or were they real-time collected stools?

DR. STERN: There are separate parts done on the test. It was done on fresh stool. It was done on frozen stool. It was done on frozen in broth. It was done in all manners. I am happy to give you the product insert.

DR. TARR: And are there any data that can be entered into our analysis or our assessment of the test as we look forward to putting it on line in terms of a trial?

DR. CLEARY: Well, as I say, it has already been licensed and it is available commercially

actually as of a couple of weeks ago. So, we are using a test that has been compared to the pre-EHIC ELISA and is equivalent. So, it is already licensed. It is already available. I am not sure what exactly--

DR. TARR: There are good tests and there are bad tests, and it might be still worthwhile for us to make a decision based on how reliable the population was, how reliable the specimens were.

DR. CLEARLY: Well, stool cultures are going to be done to look for STEC in the cultures to try to backup that and make sure whether it was an 0157 or non-0157. So, work is going to be done to address that very specific question, although not specifically to prove that assay should be licensed because that has already happened but, rather, just gathering additional backup for documenting what the patients actually have had.

DR. TARR: What proportion of the stools were frozen and what proportion were fresh? Do you remember that?

DR. STERN: What I can tell you is that the

data is separated for each section. So, the sensitivities and the specificities I gave the group before were for each specific group. I didn=t even talk about the frozen sensitivities and specificities. It does drop some with frozen stool. There is degradation of toxin, and we know this, but that is with every assay.

DR. CLEARY: With fresh stool it approaches 100 percent sensitivity and specificity as compared to the pre-Meridian EIA, the gold standard.

DR. STERN: In the V-Tech abstract from last year that will be turned into peer review, it performed actually better than the EIA methodology. That was the conclusion of this paper.

DR. RELLER: Dr. Cleary, we have five questioners in the cue. The next one is Dr. Rappley.

DR. RAPPLEY: Thank you. So, do I understand then, in terms of your response to the question about the role of standard medical care, that you are proposing 50 different sites around the world. How would you standardize the basic

supportive care and the hydration and the nature of the hydration that these children would receive? How would you factor in the public health issuesB-access to clean water, to a clean food source? And, how would the company address the notion of the ethical questions about not only the risk/benefit to the particular individual child but to the population in which this medication would be tested?

DR. CLEARY: I probably should have been writing this down. The first question was--

DR. RAPPLEY: Standardization.

DR. CLEARY: Standardization. There will be a procedural manual and each site will be trained in the use of it. Hydration will be specifically addressed in that so that standard measures of whether or not the kids are dehydrated or not will be used to make sure that adequate fluids are being given. Your other question was about?

DR. RAPPLEY: Public health measures.

DR. CLEARY: I am not sure what you mean by

public health measures here because this is a randomized, controlled trial between groups of kids who are getting the intervention. I am not sure how that is going to impact. You mean are we going to go to the public health authorities and say we have found a case of STEC and you should investigate? Is that the sense you mean?

DR. RAPPLEY: If you are working in 50 sites around the world they may have different water sources, food sources that would have different levels of contamination for the child, for the family, for the community, and how will you address that?

DR. CLEARY: I am not sure that once the kids get infected, you are going to have, hopefully, enough kids from each site that whatever the differences were in inoculum size or source of infection are presumably going to be equalized in a randomized study. This is a disease that occurs with very, very low inoculum. In the outbreak in the Pacific Northwest some years back the amount of organisms consumed in an infected burger surely was

less than 100 organisms. So, it is a very, very low inoculum disease that causes very severe illness. I am not sure that a child who takes 10 times that much--other than perhaps having a more rapid evolution, I am not sure that that is the issue. I think that the ability to look at the severity in this sort of scoring system--as long as you have enough patients from each site, hopefully, that will even out. I understand that if you only got one patient from site X and 20 from site Y you could have some issues, but I am not sure how you deal with that other than by randomization. What would you propose?

DR. RAPPLEY: Well, I was thinking about reinfection and there will be different culture milieus and how family will participate in the care and degrees, and how that exposure could be ongoing rather than just episodic or occurring at one point in time.

Then, my third question had to do--

DR. CLEARY: Well, let me just comment on that.

DR. RAPPLEY: Sure.

DR. CLEARY: These infections, even in places like Argentina where they are incredibly common, still aren=t events that occur every day. Although, certainly, you can get contaminated juices, contaminated water, you know, the likelihood that they are infected multiple times I suspect is low, although I don=t know any way to know that anymore clearly. I am sorry, the other question you had?

DR. RAPPLEY: It just had to do with the general question about trials in populations outside of the U.S. where populations may not then have access to the treatment in the future.

DR. CLEARY: Yes, I don=t know how that issue is going to be dealt with. I don=t know if someone from Thallion wants to address the issue if we do the study in a place where they can=t subsequently afford the medicine, what do you do?

DR. RAPPLEY: And is that a different risk you are asking an individual to take on? It is a risk that is beyond that for the individual child;

it is one for the population.

DR. CLEARY: Right, for the individual child, hopefully, the intervention will represent something that is good for him. I see what you are saying, are you then going to use that population to figure out your medicine works and then not sell it there, or sell it at a price where nobody could buy it?

MS. MELL: My name is Maria Mell, from Thallion. The only comment that we have on this is the fact that for any clinical trials we are going to go through regulatory agencies= approval, and it takes approval. So, the study will be approved in the country where the study will be conducted.

DR. RELLER: Dr. Edwards?

DR. EDWARDS: Assuming 50 patients per month as an accrual rate, could you give us an estimate on the number of patients that would need to be screened globally from the data you have at this point?

DR. CLEARY: The data we have at this point I think would suggest that aboutB-well, it

obviously depends on where you are doing your screening. In the United States, the further north you go, obviously, the less patients you need to screen to get one who is infected. If you go to Argentina you may need to screen very few patients to get a positive. So, it varies tremendously geographically. The frequency in the United States is likely to be in the order of about 1/100 or a bit less. But that is going to depend a lot on what the criteria are for screening. In other words, Dr. Tarr has talked about a lot of ways to improve your odds that your screen is going to detect infected kids--clinical profiling, what sort of illness does the child actually have. So, the projection is based on the assumption that it may be as bad as 1/100 but clinical profiling, seasonal screening, not doing screening in the middle of winter when most of what is coming in is rotavirus, focusing on late summer, early fall at the various sites, we hope to do much better than 1/100 but we have been planning based on a frequency of 1/100.

DR. EDWARDS: I wonder, similar to the

importance of the diagnostic test in the entry criteria is going to be the validation of the STME since you are anticipating not reaching statistical significance, and it seems to me that that validation has to sort of go both forward and backward. By that, I mean the validation for how the STME predicts HUS and then how the therapy, which may prevent HUS but may not have activity against the other components of the tool. So, I just wondered what the plans are--

DR. CLEARY: Yes, I think one of the inevitable problems with this kind of study is that at the end of the day, if you have statistical significance, then you have to look and say, well, what difference did it make. You know? If you end up with the same number of HUS and the same severity of HUS and on average one day less hospitalization who cares. I mean, basically it is very difficult to predict whether or not statistical significance is going to end up with something that everybody agrees is clinically important. If it ends up that you dramatically

decrease hospitalizations and you dramatically decrease the frequency that the kids need to be dialyzed, well, that is a big deal. But that dramatic statement, accompanied by a statistically significant decrease in events would, hopefully, be enough to help everybody judge whether or not the study has adequately addressed the concerns that we all have about it.

DR. RELLER: Many questions and increasingly less time. Dr. Daum was next, and then it will be Dr. Griffin and Cnaan, Rosenthal and Wong-Beringer. Anybody who wants to take a pass if the question has been answered is free to do so.

DR. DAUM: Mine hasn=t been. Hello, Tom. I haven=t seen you for so long I didn=t recognize you! My question goes to-BI guess we have become armchair experts this morning with the wonderful presentations that we heard and I was particularly struck by the presentation by Dr. Nostrandt, who talked about the mouse model and pointed out that there were no lesions seen in the glomerulus, that

they don=t develop hemorrhagic diarrhea, and that the cytokine production was quite different than what happens in people. You, in your handout, said that the monoclonal protects animals in a murine toxemia model.

DR. CLEARY: Right, from death, from death that is induced by toxin.

DR. DAUM: Okay. So, the question is, is there any way to have a peek at the data but, secondly, what about the mouse model as the choice of endpoints to bring to this committee? It sounded like the piglet might be something you would want to do before you started aggressively thinking about humans.

DR. CLEARY: First just to talk about the kind of data that is in the mouse model, basically, in mouse models thereB-there are, broadly speaking, with the animal models two kinds of models, models where people [sic] get toxin, models where infection is induced and then a series of events occur.

[Slide]

This just shows the ability of the antibody to neutralize when given after infection with a toxin 2 producer. Okay? So, this is proof that you can give antibody after infection is already under way, after the events that may lead to death. It is not HUS. The truth of the matter is no model really adequately models human HUS. The problem with the piglet model-Bit is a very good model, there is no question, in many, many ways. That is a model that has been done for about 20 years in Saul Tzipori=s lab.

There is one study that is also published that we heard about this morning that was not from Tzipori=s lab. That one study said there are glomerular lesions. Tzipori says in 20 years of doing a model he has never seen anything that looks like glomerular lesions.

So, there is some debate about whether that model actually perfectly mimics HUS. That is really a model that, in a sense, is like the mouse model. It is a model of neurologic injury, not of glomerular injury. It may be still relevant in the

sense that they are both models that look at toxin-mediated injury to cells and what that does to the animal. So, in a broad sense they have some relevance.

The problem with those models in terms of trying to evaluate a monoclonal antibody intervention is that to get disease, for example, when Saul Tzipori injects an 0157:H7 strain, strain called 8624, he has to give 10^{10} organisms to the piglets to cause disease. The problem with that is that in human beings you are probably getting 10^{1} or 10^{2}. The pace of disease is very different in the piglet than it is in the human. It is very difficult to know what any monoclonal antibody intervention in that setting would be. He has shown that antibody given in that setting even after diarrhea has begun prevents death, much like this data in the mice that we are showing, but, again, it is not precisely an HUS model and it has this inevitable problem that all of the animal infection models have, and that is the need to give a dose of organisms so out of proportion to what

human beings see that the pace of infection is incredibly different and you have no way to know what any kind of antibody intervention would mean.

DR. RELLER: Dr. Griffin?

DR. GRIFFIN: I just have a simple question, perseverating on the Biostar. Just assure me that the studies by Biostar were with human natural specimens; they weren=t with spiked stool. Right?

DR. STERN: That is correct.

DR. GRIFFIN: Okay. And, I heard the test is highly sensitive and specific. Can you tell me the false-positive rate?

DR. STERN: According to the PI, the only time that the test showed positives that another method did not was with SMAC plate, and in those cases it was confirmed to be non-0157 strains so the Biostar test was correct. The SMAC just couldn=t pick up the non-0157s.

DR. CLEARY: So, it was actually a true positive, not a false positive.

DR. STERN: Yes.

DR. GRIFFIN: A couple of other comments, questions, and they relate to if this study should go forward, in your study design I can understand your point about using standard of care for hydration wherever you are doing the study. Would you be collecting detailed information on hydration and on sodium administration?

DR. CLEARY: We actually have not prepared a detailed protocol yet. I think that is a perfectly reasonable thing to have in such a detailed protocol so I think that would be appropriate to do. Certainly, there are likely to be some variations in fluid administration, on matter how we hard we try to make sure everybody is hydrated, and that would be information that might be useful too. I think the observations that Dr. Tarr has made are very, very important.

DR. GRIFFIN: Yes.

DR. CLEARY: I don=t know that they are gospel yet. I mean, I think they are important. Hydration is important. Whether fluid load, in the sense that you may cause pulmonary edema, that is

harder to know that we ought to be taking that risk--

DR. GRIFFIN: Right, it is that lack of knowledge that I am speaking to--

DR. CLEARY: Right.

DR. GRIFFIN: B-because if it turns out that hydration or administration of sodium is the best predictor for HUS, then if you do not include that in your analysis in both arms, then your whole study will--

DR. CLEARY: I think your point is a good one--

DR. GRIFFIN: Yes.

DR. CLEARY: -Band it is clear that we ought to be collecting that data--

DR. GRIFFIN: Right.

DR. CLEARY: B-to make sure that we can analyze it.

DR. GRIFFIN: Right. And, similarly, data on antibiotics and which particular antibiotics.

DR. CLEARY: Right.

DR. GRIFFIN: And one other point speaking

to the scoring system is that I think there are multiple scoring systems that we could think of that may or may not be useful, but you are describing a study here in which, in your placebo arm, you will have the information to create a scoring system. So, it could be that you could bootstrap on your study the evolution of a better scoring system.

DR. CLEARY: It is certainly possible that you end up with a better scoring system as you go along during this. The reason that we like what Dr. Bitzan has done is that, for example, his enteropathy score seems to relate to something important, namely, duration of hospitalization. So, even that indicator, which is the one that we thought was most debatable in terms of a score or something you might have impact on, looks like it probably is relevant. So, yes, the scoring system needs to be fine-tuned and reworked and thought about more and, you know, I think the criticism is perfectly valid, but I also think the scoring system, as he has defined, is one that if you show

a dramatic difference, statistically significant difference with clinically relevant improvement associated with the arm that gets the monoclonal antibody, I think that is going to be something that will be of interest.

DR. RELLER: Dr. Cleary, the five questions became seven and we are going to close it at that for the next presentation. So, we have Dr. Cnaan, Rosenthal and Wong-Beringer.

DR. CNAAN: I actually have just one question and that has to do with the Phase II. You start with a 1 mg/kg. You give it to 15 children. So far your experience is only in adult healthy volunteers, and after 15 children you triple the dose. I wonder whether 15 is enough to then jump to three-fold.

DR. CLEARY: Right. In adults the doses that have already been evaluated actually are in the range all the way up to 10/kg. So, the 1 and the 3 have been evaluated, as well as higher doses, without evidence of significant toxicity. So, the prediction is that whether we are doing 1 or 3 in

children, they are both likely to be very safe. But it is important to be sure that, in fact, that is true. Obviously, these are relatively small numbers and you could argue, well, all you are going to pick up is major differences. True, all you are going to pick up is major differences, but if there are major differences that is good enough reason to change what you are doing, to go with the lower dose perhaps.

DR. RELLER: Dr. Rosenthal?

DR. ROSENTHAL: I just have a quick question and maybe the answer to this question lies somewhere in our lack of a complete understanding of the relationship between Shiga toxin and hemolytic uremic syndrome. But since you are planning on enrolling kids with diarrhea, whether it is bloody or not, and then, as a second step of the enrollment, evaluating them for toxin and we know that not all those kids go on to develop the outcome that we are talking about preventing, what percentage of the kids who are enrolled in the study are you imagining will be treated

unnecessarily? What percentage of kids do you think are not even at risk for the outcome that we are talking about measuring?

DR. CLEARY: Using the Biostar, the data are correct that are out there, and with the basis of it being a licensed test, we are not likely to be treating anybody unnecessarily in the sense that they are all going to have toxin-related diarrhea. They are all presumably going to be at risk for toxin-mediated events. Now, it may turn out that in an outpatient setting you are picking up a milder spectrum of disease because you are using a test that is picking up perhaps more non-0157s. That is certainly possible. I think that is a really important issue, that you may end up with a lot of non-0157s and that is something that will need to be looked at because, if it turns out you are picking up too many non-0157s who weren=t due to get very severe disease, you have to change the enrollment scheme to make sure that there are enough 0157s and you have enough bad outcomes to be sure you are making a difference.

DR. ROSENTHAL: So, that really is the key to my question. Is this really a proposal to treat people who manifest the toxin, or is it a proposal to treat people who are at risk for this more serious outcome?

MR. CLEARY: I think it is a proposal to treat kids who have organisms that are making Shiga toxins and are at risk of adverse outcomes that we know are associated with Shiga toxins. Even the kids who have non-0157s may end up with hemorrhagic colitis and hospitalization. Decreasing the severity of that and preventing hospitalizations, even if those kids weren=t ultimately at risk for HUS, may still be an important outcome, depending on cost; depending on toxicity. If it ends up being a minimal or little toxicity issue and you prevent a lot of hospitalizations it still may be a worthwhile intervention in those kids. But I think it is absolutely true that using a sensitive test as your enrollment criteria you may well end up picking up lots of non-0157 STEC and that is an important question and we will have to see.

DR. RELLER: Dr. Wong-Beringer, you still have a question?

DR. WONG-BERINGER: Yes. I wonder do we know whether different strains produce different levels of toxin, and if they do produce different levels how would that affect the kinetics of the drug in vivo given the same dose?

DR. CLEARY: Right. Dr. Acheson and others have shown that different strains do make hugely different levels of toxin, and that may be an incredibly important variable in terms of which kids actually get into trouble. It is not fully defined yet but it is likely to be important. I think the key thing here is that you have antibody that has a half-life of nine days. You are likely to have it hanging around long enough to continue neutralizing should you have a higher level toxin than you anticipated. The dose is calculated to be ten times the dose needed to kill a mouse on a per kilo basis. So, you are likely to have pretty good antibody excess and likely to have that antibody excess for a long period of time.

DR. RELLER: A final question for Dr. Cleary. Dr. Fant?

DR. FANT: Yes, Tom, this is a general question related to antibody therapy. What is the recurrence risk of HUS?

DR. CLEARY: With Shiga toxin-producing bugs the recurrence risk is close to zero. In other words, if you get HUS one time you appear to not get it a second time, as opposed to, say, complement associated effects that cause HUS, or whatever.

DR. FANT: Right, and the reason I am asking, I mean, is it conceivable that antibody therapy would modify the patient=s immune response to the Shiga toxin that may predispose them to an increased risk of recurrence, and would exposure to the antibody trigger a separate immunologic response that will make antibody therapy less beneficial in a subsequent course? This gets back to Dr. Tarr=s point where if hydration does the same thing, it preserves their immunologic response.

DR. CLEARY: Right. First, I think the issue is that immunity at the gut level is more complicated than just making serum antibodies, that you are making antibody to entemin [ph] and to the other proteins that are involved in the attachment of the organism to the gut, and that development of those antibodies, IgA at the gut level, is probably more important in terms of protection when you subsequently see a related bug than perhaps anti-toxic immunity which doesn=t develop quite so predictably in everybody. So, I think that the risk of impairing development of immunity for subsequent exposure is likely to be low or non-existent. Therefore, the risk that a second episode and that they might have had some immune response that now the second administration outcome this drug would be less beneficialB-I think it is theoretical risk because there is a small subset of patients who develop anti-chimeric antibodies who might have a more severe reaction, for example, or more rapid clearing of the subsequent exposure. But the likelihood that multiple exposures to these

products would occur should be very, very, very low.

DR. RELLER: Thank you, Dr. Cleary. Our second and final industry presentation will be by Teijin America, Inc., Drs. Brookman, Cato and Peterson in succession.

Teijin America, Inc.

Strategy, Issues and Alternative Approaches in Development of a Treatment for HUS Prevention

DR. BROOKMAN: I would like to thank the advisory committees for an opportunity to present today. By the way, it is Teijin, and the Japanese translation of Teijin the Emperor=s fake silk because we started off as a rayon company.

[Laughter]

[Slide]

Today I am going to talk about strategy issues and alternative approaches in development of a treatment for prevention of HUS.

[Slide]

Our working framework for our development was that we believe that clinical outcomes are a

primary source for safety and efficacy, but we also believe that there are limitations in the availability and utility of clinical outcomes and, more importantly, we think that clinical outcome data needs to be supplemented with nonclinical data.

[Slide]

Today I am going to outline potential data sources for establishing safety and efficacy of agents to prevent HUS and to show how data from nonclinical sources can be used to supplement clinical the data.

[Slide]

The approach that we took was really to focus on the package insert and looking at traditional drug development. These six points focus on what a clinician would need to prescribe a drug, going from patient population, timing of treatment, dose, dose regimen and clinical benefit, as well as potential risks.

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So, if we look at the desired indication

that we would be looking at, it is for the prevention of HUS in a pediatric patient population with either watery or bloody diarrhea, and that is 0157 positive or SLT positive.

[Slide]

I am going to first probably review things that have already been stated today but I am going to do it in an abbreviated fashion on the clinical course of Shiga toxin-producing E. coli infection and HUS; talk about a therapeutic approach for intervention; present some potential corroborative outcomes; and then integrating the clinical and the preclinical data for evidence of efficacy and safety; and then present some conclusions and some dilemmas.

[Slide]

We have all seen this graphic ad nauseam today, no offense, and it bears a little bit of repetition. The point that I want to make on this slide is that even in the absence of bloody diarrhea patients can develop HUS and we have examples of that from Argentina data, as well,

onset of HUS can be as late as 8 days after the onset of bloody diarrhea in our hands.

[Slide]

Very quickly, we have all seen this, what are the current supportive therapies. We have talked about the problems with antibiotics and antimotility drugs. We have discussed hydration, dialysis and plasma exchange.

[Slide]

So, the approach for therapeutic intervention that we have taken is really to look at what is traditional drug development and how have we fit our program into the traditional drug development.

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The first point is that we need to target a critical step in the pathophysiology of a disease. We need to analyze the protective effect in animal models. We need to assess the safety and PK preclinically, and then move into a healthy volunteer environment looking at safety and PK and, finally, getting into safety and PK in the expected

patient population and, finally, to conduct the key statistically significant randomized clinical studies.

[Slide]

So, today I am going to present the Teijin data to illustrate the practicality of this approach. First step, targeting the critical step in the pathophysiology of the disease. Just to bring up the point, I have TMA-15 listed. It is a humanized monoclonal antibody that is directed against the B subunit of SLT-2, SLT-2 being critical for the majority of HUS cases. TMA-15 binds and neutralizes to the SLT-2.

[Slide]

Our first experiment that we did was an in vitro experiment where we took STEC clinical isolates. We took 200 STEC isolates and did neutralization testing and were able to neutralize 198 of these isolates at 10 mcg/mL of TMA-15. Based on that, we established our target concentration for TMA-15 as being greater than or equal to 10 mcg/mL.

[Slide]

The next step in the development plan was to analyze the protective effect in animal models.

[Slide]

This particular overhead talks about an SLT-2 induced mortality model in the mouse and we are looking at survivability and looking at mouse survival when mice were injected with 10 times the LD50 of SLT-2. Using a dose of 1.23 mcg per mouse, we see 100 percent survival and decreasing survival with decreasing dose of TMA-15, with complete mortality in the placebo group.

[Slide]

Moving to a STEC-induced mortality mouse model, we looked at 10-day survival with administering TMA-15 as a single dose 24 hours after inoculation of STEC. On this overhead we see complete protection at the 1 and 2 mg/kg dose level, and decreasing survival at lower doses, with complete mortality with placebo.

[Slide]

This slide shows SLT-2 concentrations at

48 hours in STEC-infected mice where we see peak concentration at that 48-hour time point. If we look at the 1-12 we don=t see any SLT-2. We start to see a rise at 24 hours, peaking at 48 and then starting to drop off at 72, and we are unable to detect it at 96 hours.

[Slide]

Then when we look at mortality when TMA-15 is administered up to 24 hours after the challenge with STEC, and this gives different time points, 1 hour before, 12 hours and 24 hours afterwards, as well as 48 hours afterwards, most survival can occur up to the 24-hour mark but not at 48 hours. This was done at a TMA-15 drug level of 2.5 mg/kg.

[Slide]

If we superimpose the SLT-2 data with mouse survival we can see that at 48 hours, when it is peaking, we don=t have any survival.

[Slide]

The next step in the development was to assess toxicity and pharmacokinetics preclinically.

[Slide]

We have completed most PK/PRODUCT, mouse toxicity, monkey PK, monkey toxicity cross-reactivated with the human tissue panel, as well as we are now conducting a series of piglet studies.

[Slide]

In mice that were administered doses up to 2.5 mg/kg no toxicity was noted. The half-life was approximately 14 days. In monkeys where we administered drug in exaggerated fashion, up to 20 mg/kg, there was no toxicity that was noted; half-life was slightly below what we saw in the mouse, around 12 days, and there was no anti-TMA-15 antibodies that were observed. In addition, when we looked at human tissue cross-reactivity for TMA-15, we observed none for an extensive panel.

[Slide]

This PK graphic is just given for illustration at the 20 mg/kg dose where we see that the drug remains well above the 10 mcg/mL for an extended period of time and there were no differences between male or female monkeys.

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The next step that we proceeded to was to assess safety and pharmacokinetics in a healthy adult population.

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We conducted a randomized, double-blind, placebo-controlled study in healthy adults. We gave a single IV infusion and 24/32 subjects that given the IV infusion received TMA-15 at doses ranging from 0.1 up to 3 mg/kg.

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The results indicate that there was dose proportionality based on Cmax and AUCs. The terminal half-life was consistent across the doses, which ranged I think from 25 to 29 days, and mean plasma concentration remaining above the 10 mcg/mL level was approximately four days for the 1 mg/kg dose level and more than three weeks at the 3 mg/kg level.

[Slide]

In terms of safety, there were no AEs that were considered related to the TMA-15 and there was