cohorts. Indeed, about 10 percent of cases in outbreaks are secondary. However, it is also important to remember that the vast majority of an index patient=s contacts will not become secondary cases so an awful lot of people would have to be treated or randomized in the sphere of an infected child to find a few children who are secondary cases and, as Dr. Griffin noted, the median number of outbreaks in a secondary case would constitute an outbreak by CDC=s criteria as 5. So, there is not a tremendous number of people who will secondarily get E. coli 0157:H7.

Finally, massive outbreaks tend to be on the wane at the time of discovery of these outbreaks. Very few people are incubating an E. coli 0157:H7 infection when an incriminated vehicle is identified and then withdrawn from commerce. So, that would not be such an easy group to target and then treat.

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But let=s say you do have a contact of a known case, and let=s say you really do think you

are in the midst of an epidemic, and you really do think you can implement an intervention, mostly in the context of a trial, assuming that you had all logistic and human subject obligations met and you were able to get patients randomized, in this situation there are some limitations because there are probably strain to strain bacterial variabilities in the risk of developing HUS. So, any lessons learned from such an endeavor would probably be biased towards strain-specific findings and may not be generalizable to diversity of strains encountered looking forward.

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Again, let=s look at the time line. You have 3 days of incubation, not much you can do there. Prior to a patient presenting for medical attention, not much you can do there. You might, at that point, by augmented profiling, by better microbiology, increase the number of diagnosed patients and bring them sooner in the time line. This would be analogous to a better test at identifying a patient with myocardial ischemia in

the emergency room rather than waiting for the serial enzymes over the next several days of hospitalization. You might be able to do a little bit better at that point, and you might be able to intervene and study.

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Unfortunately, not only is not time in your favor, this is a rare disease. By Dr. Griffin=s estimates, there are well under 10,000 diagnosed cases of E. coli 0157 in the United States I-scaled up for modeling purposes. If we could triple the number of patients we could diagnose with improved diagnostics, with better awareness and we come up with 10,000 diagnosed cases per annum. About 25 percent of them will be in the prime HUS risk factor group under age 10. That means that there are only going to be, on an annual basis, 48 patients per week under age 10 who will develop the first symptoms of an E. coli 0157 infection, and only 7 patients per week or 1 child in the United States per day will become symptomatic and then develop HUS.

That means that right now, today, there are 3 or 4 children in the United States who have already presented to a doctor in the past 24 hours who will go on to develop HUS in the next week. This is a very low number to identify when you consider a population in excess of 300 million. Additionally, there are 14 children in that cohort who, if they could be identified, will not develop HUS meeting our case definition as described earlier. So, these are going to be the numbers in modeling any sort of an intervention trial.

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Let=s put under the microscope that critical first encounter, and to do so I am going to use the example of the Children=s Hospital and regional medical center study conducted by Dr. Eileen Klein. This is a high acuity emergency room. It is a prime point of care. It saw more children in the 1993 outbreak than any other center in the United States. Everybody there is quite aware of the virulence of this organism. There have been multiple research projects focusing on E.

coli and bloody diarrhea and HUS in this institution. Everybody knows about it. Everybody is alert for this organism. There is also an outstanding microbiology laboratory located 100 yards from the emergency room. This is a great opportunity to see how this organism behaves in populations reporting to one central node.

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When Dr. Klein did this study she filled out a prospective questionnaire for all enrolled subjects in the study, but also we culled from that the children who had E. coli 0157. The median number of stools in the 24 hours prior to coming to the emergency room was 7. That meant that there was a median inter-defecation interval of 206 minutes. Assuming an emergency room visit of 4 hours, which some people would say is not bad in an urban emergency room, that means that by a Poisson distribution there is only a 70 percent chance of producing an analyte on site. You had better really profile these kids well and not wait for the microbiologist to tell you that you have a problem.

I personally think all such patients should be admitted.

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In this 3-year window nearly 5,000 children came in with diarrhea or gastroenteritis and were offered the opportunity to enroll in this study around the clock, 24/7. Of these nearly 5,000 children, we enrolled only one third, and enrollment was defined as filling out a questionnaire and allowing us to get either a cup specimen of stool from the infected child or getting a rectal swab on the infected child. So, this too demonstrates the challenges of studying enteric infections in North American populations. It is hard to get samples. Of those 1,600 child, 39 of them produced a Shiga toxin-elaborating E. coli. As I said before, 28 of them were E. coli 0157. Every 39 days on average, more July through October, less this time of year, a patient came in with an E. coli 0157 infection. Five children during these three years subsequently reported to this emergency room early in illness, subsequently

developed hemolytic uremic syndrome. That meant that in the 15,000 sq. ft. emergency room, which is one of the highest densities of presentation of children with E. coli 0157 in the United States, once every 219 days a subject came in who then went on to develop hemolytic uremic syndrome.

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Now, we could obviously profile some of them by listening to their story. There may be a few other ways to profile them. Of the 1,626 patients who allowed us to have a stool culture, 1.7 percent of them were positive for 0157. That is a reasonable percentage. But of children who submitted stools in a cup, usually representing children somewhat older, outside the diaper range, 5 percent of those children were positive for E. coli 0157. So, that is a potential target for study. Of children who didn=t submit stools in a cup where we were forced to swab the child=s rectum or a diaper, 0.6 percent were positive. So, it is possible that you can do a few things in addition to looking at the calendar, but you can do a few

things to weight towards findings positives and a cup specimen might be one way.

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Bloody diarrhea in the setting would be another way. This is again from the 3-year study by Eileen Klein. Nearly 10 percent of children who came in with a report of bloody diarrhea, parental reports spontaneously completed on the questionnaire in the waiting room before any questions were administered by a provider, there was nearly a 10 percent positive rate for E. coli 0157 in this setting. Had you used bloody diarrhea in this setting you would have found 22 of those 28 infected patients with E. coli 0157. You would have missed 6. Once every 7 weeks a patient would have come in and been potentially entered into the study. However, had you used bloody diarrhea as your index and HUS as your outcome, you would have enrolled a patient only once every 39 weeks who subsequently developed HUS.

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One of the big questions here is when you

get these patients in this earliest encounter in the healthcare setting is the microbiological horse out of the barn? Is the host injury already under way? This is going to remain an open question. But what we have tried to do is identify children and get blood and urine from them on or before day 4 of illness, quite early in the cascade as far as the kinetics of presentation go, probably about the fifth inning in terms of the whole game. But this is the first opportunity to get them. This is their first encounter with the healthcare system.

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When we looked at an index of thrombin generation, the ability to generate fragment 1 plus 2, we found that prior to the development of hemolytic uremic syndrome, and even in children who didn=t develop HUS, there was activation of the coagulation system suggesting extra-intestinal injury had already begun. Again, on or before day 4 of illness we found evidence of elevated levels of circulating D-dimer in the bloodstream of these children, higher levels in the children who

developed HUS, not quite as high in the uncomplicated courseB-too much overlap to use this as a differentiating characteristic looking forward. D-dimers represent intravascular fibrin accretion, suggesting that thrombi were already present in these children on or before day 4 of illness, at a point of illness when children had absolutely normal hematocrits prior to the development of HUS, or in the group who didn=t develop HUS absolutely normal platelet counts and no evidence of functional renal impairment. So, again, we have activation of the coagulation system prior to the development of renal injury that we could demonstrate at a point where there is 10^7 or 10^8 0157's pre gram of stool where there is not much toxin left in stool.

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These are the data from Nancy Cornick. Twenty percent of children prior to the development of HUS had detectable toxin in their stool. But half of the children who didn=t develop HUS had detectable toxin in their stool. At the time of

HUS the toxin is just about gone from this population. The titer, paradoxically, was lower in the children who subsequently went on to develop HUS.

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There are a few other items that I would like to mention parenthetically. Several years ago there was a flurry of interest in polymorphonuclear leukocytes as the shuttle vector for toxin. White cells would take toxin from the gut and deliver it to the kidney. However, in a recent publication from The Netherlands, the group that initially reported this, now states that polymorphonuclear leukocytes are not acting as transporters for Shiga toxin in the pathogenesis of childhood HUS.

There is elevation of platelet activating factor in the plasma of children prior to the development of HUS on or before day four of illness. Platelets are activated possibly by the E. coli 0157 lipopolysaccharide and von Willebrand factor also shows evidence of shearing prior to the development of HUS, before there are any

discernable classic hematologic abnormalities, suggesting or raising the possibility that there are already thrombi that are causing rheologic abnormalities as von Willebrand factor goes through small vessels.

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Then let=s go back to our patient cohort. If one did have an intervention, what should we do? Even in the absence of an intervention, what should we do now? Well, obviously, we want to avert the case definition of HUS and send a patient home as rapidly as possible. Using a fairly stringent case definition that includes functional criteria for renal impairment, namely, elevated creatinine and not an abnormal urinalysis which can be a very difficult and often flawed specimen to obtain in the setting of diarrhea, we sorted out the groups of children who went on to develop HUS. So, we said there is HUS and then there is severe HUS.

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Now, mild HUS requires transfusions in

almost all cases. Ninety percent or more of such children with mild HUS will require erythrocyte transfusions. It does not require dialysis and children are not anuric. The length of stay in this cohort of a 6-year study that we performed prospectively at the Seattle Children=s Hospital among all children with HUS, all of whom were culture positive for E. coli 0157, the length of stay was only 6 days.

Then there is a categorically different form of HUS, that requiring dialysis. Children are anuric. The model for this is actually acute tubular necrosis. When they stop urinating they generally stop urinating for 4 or 5 days at a minimum. The length of stay in this group is 12 days.

Several studies, first from Dick Siegler at Utah and more recently a meta-analysis published in JAMA, suggest that the need for dialysis, which is a surrogate for anuria largely, is the biggest single predictor of chronic sequelae. So, if a patient develops HUS it is much better to be in the

non-anuric group.

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However, it is separated in pre-HUS care and pre-HUS events. What were the distinguishing characteristics of children who went on to develop anuric HUS compared to those who developed non-anuric HUS using an intervention that has been available for some time, namely, admission to hospital and vigorous hydration? Those in this cohort who were positive for E. coli 0157:H7 were enrolled via variable routes, which I suggest could be a model for subsequent studies of an intervention. Two of these 29 children were identified prospectively by profiling them. They came into the emergency room. They had bloody diarrhea. They fit an E. coli profile. They were admitted. They developed HUS. One of them was a household contact of a patient with E. coli 0157:H7. Nineteen of them entered the study because a microbiologist made a presumptive diagnosis of E. coli 0157. This is the most critical event in the course of a child=s illness.

Seven children arrived at the hospital with established hemolytic uremic syndrome, not having been pre-diagnosed microbiologically.

We interviewed families immediately. We got all hospital records. We asked for all possible pre-HUS events that could be plausibly related to this outcome. It is kind of like looking at people who survive myocardial infarctions versus those who don=t and saying when did you get care; what did they do?

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In this slide which is somewhat complicated but is actually fairly simple in this concept, we said who had bad outcome anuric HUS? Those people are represented by the open white bars. Who had good outcome? Those people are represented by the grey bars. Children who had good outcome presented to medical care a little bit earlier than children who had poor outcome. They had a stool culture obtained 24 hours before children who had bad outcome. The first IV was started one and a half days before in the good

outcome group compared to the bad outcome group. That doesn=t sound like a lot, except this is a disease that plays out over a week. That is a sizeable proportion of time. They were tested with white counts and creatinines a day or two in advance of the bad outcome group. And, the first culture we reported to the provider as being presumptively positive was 2.5 days sooner in the good outcome group. So, when the microbiologist gets on the phone and let=s the primary care provider know or the emergency room provider know that an E. coli 0157 is under consideration, that child is probably going to be handled better. It is hard to prove this prospectively in a randomized study, but a child who is microbiologically well assessed does better.

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We then asked what intervention could possibly have separated the good outcome from the bad outcome group among these 29 children. Children who had anuric HUS received one-tenth of the amount of intravenous volume and one-tenth of

the amount of intravenous sodium in the first four days of illness, well in advance of microbiologic diagnosis in about half the cases. Sometimes it was at the time of microbiologic diagnosis. This is almost always in the form of emergency room administered fluids.

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When we looked at all the potential factors in a multivariate analysis, the amount of sodium received in the first 4 days of illness was the biggest determinant of who developed anuric HUS versus nonanuric HUS in this cohort.

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So, getting back to the three-year emergency room study of Eileen Klein, once every 7 weeks a patient came in with E. coli 0157. Once every 39 weeks a patient came in with E. coli 0157 who subsequently developed hemolytic uremic syndrome. In this 3-year interval not a single child came into this emergency room who subsequently went on to develop HUS and required dialysis. They stayed a median of 6 days, required

transfusions, kept urinating and went home.

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We now have four distinct patterns of outcome in this illness. Pattern one is the best outcome. Patients are identified early. They are admitted to hospital. They receive IV hydration. The top left-hand panel shows what happens. The platelet count drops about 15-20 percent between the first determination and the second determination. The patient feels a little bit better on the third hospital day. The platelet count is up. We send them home. Hemoglobin, which is the second panel, remains stable. creatinine remains normal and stable.

Pattern two, which we are now seeing with increasing frequency with syndromic profiling, is also a fairly good outcome. This is a patient who had profound thrombocytopenia. We are seeing this in about 5-7 percent of microbiologically diagnosed patients now. Profound thrombocytopenia, severe anemia requiring 2 packed erythrocyte transfusions, but a creatinine that remained perfectly normal at

0.4. The child had profound hematologic injury; no evidence of functional renal impairment. And, we don=t call these children hemolytic uremic syndrome because they don=t have uremia.

Pattern three is mild hemolytic uremic syndrome, a patient with, again, profound thrombocytopenia, hemoglobin requiring 1 transfusion, a creatinine that crested at 2.2. This child was discharged from hospital several days later without dialysis. This child was detected because his sister was admitted to hospital with anuric HUS two days before his bloody diarrhea began. She was never proven to be caused by E. coli 0157. The culture was too late when she came in with HUS. She didn=t have bloody diarrhea but the sibling did, the boy did. We got his stool. He was positive. He was well hydrated. His creatinine did not rise. It is very hard to prove cause and effect though.

Pattern four is the most regrettable complication. This is a child who was in the hospital for nearly 2 months and received dialysis

for about 6 weeks; required multiple blood transfusions and actually had a myocardial infarction. He came to medical attention on day 6 of illness and his creatinine rose soon thereafter. There was not much we were able to do for him.

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In summary, if one is planning a trial, if one is planning interventions, diagnose early; diagnose accurately. Be prepared to intervene early. Be prepared to intervene early at multiple sites throughout a country. Support all patients, treated versus controls, with aggressive volume expansion. A perfused kidney is a happy kidney. Collect data that will be usable for future clinicians. We need to know when the window opens and when the window closes for intervention in an infected child. We need to define carefully what outcomes are to be averted meeting the case definition of HUS, meeting the case definition of anuric HUS which probably has the most major sequelae. All those must be weighed against cost and risk.

There are going to be important consent issues regarding urgent enrollment. As Dr. Smith talked this morning, we are neither here nor there in the subject population we are discussing. There are imperfect animal models. This is a disease which is hard but not impossible to study in a randomized, controlled trial if sufficient resources are devoted for it but it will have to be a lot of resources.

Finally, the spinach outbreak was a bit of a wake-up call in terms of toxin genotypes. It turns out that the spinach outbreak probably contains globotriaosyl that encodes Stx2c, which is an allelic variant of Stx2. It also encodes Stx2. It will probably be important to determine whether or not antibodies against Stx2 will also neutralize Stx2c.

With that, I would like to thank you. I apologize for the multifaceted, broad-ranging nature of this talk and I would be happy to take questions.

DR. RELLER: Yes?

DR. M. SMITH: I have two questions. One, if you talk about the toxin and try to compare it to other ones that we have more experience with, so if you compare it to, like, C. diff. toxin versus botulinum toxin, where is the spectrum?

Then, the second one, when you looked at the study with the laboratory at a 70 percent sensitivity in predicting whether the stool was bloody or not, was that done visually?

DR. TARR: Entirely visually. For the first question I need more elaboration.

DR. M. SMITH: When I think about Clostridium dificile toxin-related diarrhea, I know when we grab our samples, if they are not warm and hot coming to us the rate of us being able to recover the toxin is very low--

DR. TARR: Right.

DR. M. SMITH: B-versus botulinum toxin that seems to last forever.

DR. TARR: Good question. I personally filter most of those stools, obtain them from the patient, filter them at the bedside, freeze them

for further storage. So, it was warm. These were not sitting around overnight, collected and then studied. These were freshly collected.

DR. M. SMITH: I just bring that up because it means that in an average laboratory that is not going to happen.

DR. TARR: This was the best case.

DR. M. SMITH: Yes.

DR. TARR: This was a better case.

DR. RELLER: Dr. Ward, and then we will come around the table.

DR. WARD: Dr. Tarr, in the Klein study these intervals of presentation once every 7 weeks and once every 39 weeks, did those reflect simply estimates of the duration divided by cases? Since it occurs in sort of episodes during particular seasons and sometimes in clusters, I can imagine that these might be infrequent estimates if they are done in that fashion.

DR. TARR: Except for an inter-household cluster or a small daycare cluster, these were largely sporadic cases. They were

calendar-clustered. I couldn=t take summer vacations without the study suffering. This is largely a seasonal event. But, as some outbreaks have shown us, including the 1993 outbreak which was in January, it is not only July 4th through Thanksgiving.

The way we came up with the every 39-week estimate was that we said the study went 36 months, what was the numerator within that at a point that is a magnet for patients with such an infection.

DR. RELLER: Dr. Kocis?

DR. KOCIS: Just one quick question about the IV fluids, did you look at IV fluids alone or did you look at total hydration, i.e., oral rehydration? Particularly, because getting IVs in small children can be difficult. Movement, of course, is to move towards oral rehydration. So, do we separate that, and potentially did IV fluids act as a surrogate for dehydration which may be a surrogate for younger children who then come to earlier diagnosis?

DR. TARR: Age didn=t factor in so I don=t

think it was merely a technical issue of not being able to start an IV. I have often been asked, well, what about oral rehydration? Well, you know, Pedialite has about one-third the tonicity of isotonic saline. Half or more of the children were vomiting and vomiting is a risk factor for developing HUS. We were unable to reconstruct accurately what the oral intake was. We addressed that as a methodologic limitation. That would be very difficult. I do not think it is appropriate to rely on oral rehydration. Furthermore, these children often find themselves with spasms of pain precipitated by oral intake. If for no other reason than comfort, I say admit them. Let them eat and drink if they want and hydrate them.

DR. KOCIS: The second is just a comment about the enrollment issues and urgency. Being involved in sepsis in ICU patients, we can do this very quickly, very successfully. I worry about coercion with the family. Given the illness in sepsis in ICU, we worry more about coercion than I think I would in this potential trial, and stuff.

But the follow-up would be for children in North Carolina. I took care of many of the critically ill children who had that outbreak and one of them, they took a bath in the same tub and then subsequently got the two kids. But if you are going to be under incredible pressure from families to enroll siblings, as we are in a similar way with prophylaxis from meningococemia outbreaks and other things, but those are going to be sensitive issues to address in the study design.

DR. RELLER: Dr. Kaskel?

DR. KASKEL: As a nephrologist, I like the idea that you know that kidneys have to be kept happy. But in this study there are two things in reviewing the literature, which you did so beautifully. One, I am a little surprised that we don=t have a better assessment, or maybe we are not teaching some of our house staff about better assessment when a patient hits the service to see the extent of losses, whether it is by weight or physical assessment, cardiovascular assessment, some estimate of how deficient they truly are in

order to get that sodium and fluid in more rapidly.

Two, we know that before the creatinine is elevated almost 50 percent of acute injury has to have occurred to the kidney, and complicating that assessment would be pre-renal injury without the true intrinsic injury and then, once that occurs, it goes longer. In a case like this with HUS toxin we have a glomerular intrinsic injury.

So, we need a better marker than creatinine, and we are looking at that in a lot of kidney illnesses. There are some newer things on the block. N-gal galactocidase is being looked at in the blood and urine as an early marker of tubular injury and, of course, cytokine excretion which is still a mixed bag as to what that means in the urine. So, a better assessment of the injury, a more rapid assessment would be something I would like to see built into a study.

DR. TARR: I agree. We have some data that relates to all this. This is a very difficult disorder. If you were to see these children you would not make an assessment of dehydration. They

are frequently hypoalbuminemic at the time of presentation. So, any evaluation of the skin is out the door. You cannot assess the dryness. They are frequently not tachycardic. Blood pressures are unreliable in this age group. So, everything we teach our medical students and house staff doesn=t apply to this infection if they have bloody diarrhea.

The amount of stool loss is actually fairly small. I think that much of the intravascular lesion is secondary to leakage of fluid into the interstitium and the weights may even be up. When we have taken 8-, 9-, 10-year olds in whom we can clearly separate stool output from urine output, they put out 100, 200 mL of stool per day in their first hospital day at the height of illness. It is interesting, it is not cholera; it is not rotavirus on a per kilo basis. It is a vascular disorder. It is a sepsis model. It is an MI.

We were concerned about creatinine being a blunt instrument to assess renal function, and I

have been averse to getting urine now in these because they are going to have white cells and bacteria and red cells in there because they are taken from a 2-year old with diarrhea. But we have gotten a reasonable amount of urine pre-HUS and we look for beta-2 microglobulin and n-acetyl glucose and those were not elevated prior to the onset of HUS. So, we have coagulation pre-renal injury going on in this window. It is an opportunity to intervene either by looking at the bacterium and its products or potentially modulating the host.

DR. RELLER: Dr. Edwards?

DR. EDWARDS: On the basis of one of your concluding comments that this is a difficult but not impossible entity to study, and within the vagaries of the diagnostic issues and of the different patterns of the disease that have been identified, would it be possible for you to comment, based on the Klein three-year study, about how many centers might be involved to determine a specific endpoint?

DR. TARR: I did not do a number needed to

enroll or number needed to treat or power analysis. But if your endpoint is HUS you will get, in a center like this at least in the late 1990s, 5 patients in a pediatric emergency room that sees 25,000 patients per annum. Five patients will go on to develop HUS over a 3-year period. That would be the rude calculation. I can=t tell you how many will go on to develop anuric HUS because we don=t see it if they are collected in this setting. So, if your outcome is meeting the case definition of HUS, you can carry it forward from the 5 per 3 years per 25,000 visits.

For your additional consideration, my guess is the Seattle Children=s Hospital sees about 1.2 percent of all pediatric emergency room visits in the country. So, one can extrapolate forward from that.

Interestingly, in St. Louis the numbers are very similar. They are not quite so rigorously obtained but I think that this may be a potential study site. This may be a network of potential study sites if one were to construct an

investigation. But, again, 6 out of 7 of your enrollees aren=t going to have the outcome of interest, no matter what.

DR. RELLER: Dr. Wiedermann?

DR. WIEDERMANN: Thanks for clarifying a lot of issues for us. I want to go back to your syndromic profiling proposal. Basically, what you are saying is to essentially enroll patients in a study before we have laboratory confirmation that they have 0157:H7 infection. That is the syndromic profile. They come in with bloody diarrhea. They fit the syndrome. They get enrolled.

DR. TARR: That is a tough one. I can give normal saline now with little compunction. That is licensed. It is not Aunstandard@ care in many situations. Throw on top of that a novel, being evaluated therapeutic that is a dramatically increased order of magnitude. I am very interested to hear data about rapid diagnosis. That is going to be critical in these situations.

DR. WIEDERMANN: I guess my question would be, and maybe more for some of the presentations

later on in the day, if this kind of syndromic profiling is a possibility do we have something more definitive like a receiver operating characteristic curve that tells us, you know, how many patients we are going to be enrolling unnecessarily? Although it sounds like you would admit every child with bloody diarrhea to the hospital, you know, some people would disagree with that. So, I think we are proposing a major intervention if we get to that point.

DR. TARR: There is a little role for clinical judgment in this. Again, I look at the age. I look at the fever. Absence is more concerning than presence. I try to establish a time line and that is why I refined the clinical profiling. If you walk in and your fist stool is bloody, it is something else. If you have 8 days of watery diarrhea and then you suddenly see blood, you have an anal fissure. If you have bloody stools and you have diarrhea and it is true, that is not bacterial colitis.

With the profiling, it is my estimate that

2 to 3 children without E. coli 0157 are admitted for everyone who is. The admission is expensive. The treatment is probably not harmful at present. Many of the children who have other forms of diarrhea are not mildly ill. The kids with bloody diarrhea and salmonella or shigella or campylobacter, while I am delighted they don=t have E. coli 0157, are quite happy to have been in the hospital and hydrated. So, that is not extreme care. Giving a monoclonal or any other therapeutic would be an order of magnitude different consideration.

DR. RELLER: Dr. Rosenthal?

DR. ROSENTHAL: I want to be sure that I have a clear understanding of what standard therapeutic practices are as we consider alternatives to them. You have mentioned a number of supportive therapies that are used depending on what stage of illness the patient is in. And, I understand that the illness involves progression. But can you speak to any of the current therapeutic options that are available at different stages and

the effectiveness of those options, as we consider alternatives?

DR. TARR: Let me do it phase by phase. Before your first episode of diarrhea there is nothing to do. You can take heart in the fact that the vast majority of people who are exposed to E. coli don=t get symptomatic infection. The vast majority of spinach eaters probably didn=t have a single loose stool in September of 2006.

When a child has non-bloody diarrhea it is handled via telephone and oral rehydration, as it should be. There is nothing in that episode to prompt any special therapy. For bloody diarrhea it is hard to state what the standard is because there has not been a consensus conference on standard approaches but I would say that it is a medical emergency in North America, as it should be throughout most of the world. That should obligate a physician=s visit. It should obligate a good, quick, accurate stool culture and not only a toxin assay. In my opinion, if a patient is plausibly infected with an E. coli 0157 it should obligate a

hospitalization and vigorous intravenous volume expansion. When a child gets HUS it really depends on the nature, severity, duration and complication list, and a variety of subspecialties are involved. It is a multi-systemic vascular disorder, usually housed under a nephrologist service.

DR. ROSENTHAL: And I heard no mention of antibiotics.

DR. TARR: Yes, I don=t like to mention that. I don=t encourage them. There has never been a study that demonstrates that children who get antibiotics have a lower rate of developing HUS than those who don=t, and there have been several studies suggesting a higher rate. No benefit associated; worse outcome.

DR. RELLER: Miss Dokken, you had a question?

MS. DOKKEN: Dr. Tarr, in one of your very early slides where you talked about challenges and the low incidence and time being against you, you also mentioned the problem of consent in urgent situations. As I think about the number of

specialties around this table, there must be other models where there have been urgent situations, and I wonder if anybody can mention one or two and how that consent issue was addressed.

DR. RELLER: Dr. Wiedermann?

DR. WIEDERMANN: I can just speak to one trial I participated in, which was a study of steroid versus placebo adjunct therapy in bacterial meningitis. For the study we had a window of 4 hours from the time antibiotics were given to the time the steroids were given to obtain informed consent and give the steroid or placebo. Maybe much like Dr. Tarr coming in and filtering the stools, I was coming in at 2:00 a.m. to speak to families in the emergency department and enroll patients, and it was a very difficult situation. The parents had just been told their child has meningitis and all the potential sequelae. But I think, as tough as that situation was, we were able to have honest discussions about that, the risks and the benefits, even in a middle of the night in a very tense situation in an urban emergency room.

So, yes, it is difficult but it is doable.

DR. RELLER: Dr. Cnaan?

DR. CNAAN: I also have a question about the consent. In the Klein study you had a rate of about a third consent, or thereabouts. You called it enrolled; you didn=t quite say consent.

DR. TARR: We went through this with our institutional review board. We stated that if you consent to be in the study, fill out this questionnaire, and after you fill it out let us know if we may obtain stool by a swab if your child is not producing urine.

DR. CNAAN: Okay, so it is close enough to consent. So, you had about a third in a study that essentially had no risk and no benefit. If you are going to do a clinical trial, a randomized clinical trial you have some risk and, hopefully, a potential for benefit. Do you think your rate of consent, taking into account the short time line, would be higher or lower in that setup than the third you had in this study?

DR. TARR: It depends on what outcome you

are averting. If the outcome that you tell the family is that you might shorten your hospital stay by a day, many people would not take the risk. If the outcome you are trying to avert is meeting the case definition of HUS, more would take that risk. If the outcome is meeting the case definition of anuric HUS, even more would take that risk. And, if it is a situation of lifelong hearing loss, probably most would take that risk, hearing loss being the analogy for the meningitis studies.

DR. RELLER: Dr. Kocis?

DR. KOCIS: Just to respond to Deborah=s question, I think two trials come to mind. Certainly one I was referring to is activated protein C in sepsis. There, you have a critically ill child with organ failure, respiratory failure, hemodynamic failure. So, these are extremely ill children and we had a longer window to enroll than the 4 hours and, yet, we were dealing with children at UNC, again being a central hospital referring from all 100 counties, where 25 percent of our children are flown in from around the state, which,

you know, is similar to what you are saying, a rural disease that would likely come to a central center where nephrologists and other specialists are. The difficulty was in just getting a parent there. Discussions about telephone consent, we were not, by design, allowed to and I wouldn=t advocate that. Once we had the parents there, similarly, the issues of coercion and distress and what could they comprehend, and things, we had to be very sensitive about that and how we addressed that. At least in our center we did that in a tiered approach. We didn=t just go, Ahey, here=s the consent, do you want to sign? The child is going to live or die?@ You know, that had to be nuanced and couched in an extremely sensitive way.

Then, we knew up front that the drug had major complications. We knew that intracranial hemorrhage and death from that complication of the drug was there. So, this was, you know, a drug trial that we did. The trial ended up being stopped due to non-efficacy. There was increased risk for non-efficacy shown when we terminated this

study in children.

So, what I can say is it is complex. We need to think and draw a lot of input into that whole consenting process and how you are going to address this. I am less bothered by this-Bwell, not to take a potshot at anyone, but urgency for gastroenterologists is a little different from us intensivists--

[Laughter]

B-no harm intended there! So, I am not bothered by the urgency of it. I think it can be done. It can be done successfully and well and protecting patients= and family rights. So.

DR. TARR: I would like to note that one potential difference here is that your consents were obtained in a funneled area, an ICU. This was an emergency room. Even though that is a high density point of presentation, the majority of patients entered are from a five-statewide study, not via that emergency room. So, consent would have to be obtained by people at multiple different sites. That is a different order of complexity and

challenge.

DR. RELLER: It is time for the break but we want to have the final questions. Currently we do not have speakers scheduled for the open public hearing. The discussion has been pertinent so I think we should go ahead and conclude it within the next several minutes. Dr. Gorman, you had a question?

DR. GORMAN: Could you speak to the time line? The toxin identification is a little bit more rapid than the culture.

DR. TARR: An interesting question. We looked at this in Seattle and St. Louis. In Seattle until recently they plate the stools, not to 24 hours a day; they plate it to about 10:00 at night and they start again at 8:00 in the morning. The median time from submission to a presumptive positiveB-this is not a definite positive; this is a sorbitol non-fermenting organism reacting with the 0157 LPS and is not a mucoid colony, and there are a few other thingsB-was 36 hours. In St. Louis, where it is plated 24 hours a day, the times

to positive presumptive 0157 is 23 hours and 15 minutes over the past 3 years. So, the toxin assay requires overnight broth and probably once a day performance in an EIA assembly cue. That is approximately 20 hours, depending on when the specimen was received.

One of the biggest variables here, interestingly, turns out to be weekends. This is a weekend disease. You get it when you go to a petting zoo on a Saturday or a Sunday, or a barbecue. You have 3 days of incubation, a day or two of non-bloody diarrhea and it turns bloody on Friday. If your stool is sitting around, waiting for it to be shipped out until Monday you lose the window of intervention. So, it has to be 24/7 with an emphasis on Friday, Saturday, Sunday.

DR. RELLER: Dr. Tarr, you emphasized the importance of saline repletion. Given the difficulties in recognizing dehydrationB-you mentioned that term because of food loss--is there any role for looking at urine osmolality or urine sodium for recognizing that people need saline?

DR. TARR: We have not looked at that. We have the specimens collected and I wondered about looking at that but we have not looked at that previously.

DR. RELLER: Secondly, given the potential downside of giving antimicrobial therapy for the other causes of acute bloody diarrhea, is this a sufficiently important differentiation that it actually becomes-Byou mentioned the rapidity or emphasized the rapidity of recognizing this pathogenBwhat about the rapidity of recognizing the potential look-alikes, the campylobacter, the shigella so as to know when one can give therapy? Because without early therapy those entities are going to run a bit longer course as well. So, I mean, is there a triple or quadruple reason to know what you are dealing with specifically both on the upside and the downside?

DR. TARR: As you know, running a microbiology laboratory, enteric microbiologic diagnosis is balkanized. It is a technology that is 125 years old. If Louis Pasteur were to be

reincarnated today you could put him to work on your enteric expansion with a little orientation.

DR. RELLER: We have a position!

[Laughter]

DR. TARR: It is a nightmare. But if you delay antibiotic treatment by a day or two for an appropriately diagnosed patient with shigella, that is unfortunate but that child is not going to go into kidney failure. Salmonella shouldn=t be treated in otherwise healthy hosts, at least in our age group. Campylobacter, by the time the culture report comes back using standard microbiology, it has really been hard to prove that antibiotics have a role, though most of us still do treat. So, we are up against archaic technology that absolutely must be improved for us to move forward. But the 800 pound guerilla is a rare event and that is the E. coli and that happens in North America with low but year by year predictable reproducibility that we have to be aware of. Does that answer you?

DR. RELLER: Thank you. Dr. Ward?

DR. WARD: Dr. Tarr, when an outbreak

occurs it would appear to me to represent an opportunity to have an enriched sample for detecting cases.

DR. TARR: Or biased.

DR. WARD: Potentially biased, yes. But if you added your clinical profile to a D-dimer test, have you looked at that as the opportunity to have an enriched sample for detection?

DR. TARR: Well, we looked at D-dimer. In controls, it is not there. In some other children with enteric infection it is not elevated to that extent. But, don=t forget, this was a quantitative D-dimer performed in a research laboratory. If you are operating in a rural hospital or multiple different hospitals you may only get low, intermediate or high and not an exact number that you could put in. Our statisticians discouraged us strongly from using any of our pathophysiologic abnormalities that we discerned as predictors.

DR. RELLER: Dr. Acheson?

DR. ACHESON: Phil, you focused very much on the short term. Can you say a little bit about

long-term consequences? I am interested not only from your two categories of HUS, but you have 85 percent in one of your earlier slides as essentially spontaneous recovery. What do we know long term about that group in terms of potential impacts 20 years out?

DR. TARR: The best study has probably come from a Amit Garg and Walkerton who looked at patients in a Milwaukee outbreak and the effect of E. coli 0157 but who didn=t develop HUSB-no renal sequelae in several years, I forget what the exact follow-up time was. It was published about a year and a half ago in Kidney International. So, probably about a 4-year follow-up. So, I think danger to the kidney has never been established. Danger to the kidney for the long term for children who don=t require dialysis is also extremely rare. Garg also published another meta-analysis demonstrating that no dialysis is very unlikely to have any chronic renal sequelae. Dialysis is the categorically different kind of HUS, suggesting that the difference between no HUS and HUS that

doesn=t require dialysis is less, from a prognostic standpoint, than HUS that does require dialysis. That is a bigger step.

I think that there may be a reasonably large subset of patients who develop subsequent functional gastrointestinal disorders, particularly irritable bowel syndrome following this and a diversity of additional diagnosed bacterial enteric infections. That has not really been looked at adequately in different populations.

DR. RELLER: Thank you. Our final question, Dr. Rehm?

DR. REHM: Back to diagnostic testing, if you could have any diagnostic tests you wanted, what would you choose and in what time line would you say that that test might be available? I heard Dr. Griffin mention PCR.

DR. TARR: It should be before the patient leaves the emergency room or the point of presentation.

DR. REHM: I am not talking about timing of doing it, I am talking about when will a better

diagnostic test be available, and what will it be?

DR. TARR: I think it is going to be DNA-based. I think it is going to have to address multiple different pathogens. You need to assess what the microbial population is going to be. And, I am aware of several attempts to develop such technology using microfluidic flow, essentially making enteric microbiology as reproducible as getting a CBC. Who knows, perhaps in the next several years it will become available.

DR. RELLER: Dr. Tarr, thank you for fielding a wide range of questions and for your fine presentation. We will take a 15-minute break and start promptly at 11:30. Thank you.

[Brief recess]

DR. RELLER: This morning and a crucial part of this afternoon=s discussion will be the appropriate positioning or consideration of animal models in assessing potential interventions for HUS and related complications. Therefore, we are pleased that our next presentation will be a thorough discussion of experimental animal models

for the evaluation of therapeutic products indicated for Shiga toxin-producing infections. The speaker will be Dr. Amy Nostrandt from the FDA. Dr. Nostrandt?

Experimental Animal Models for the Evaluation of Therapeutic Products Indicated for Shiga Toxin-Producing Infections

DR. NOSTRANDT: Thank you.

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I would like to discuss today some of the animal models that have been published in the scientific literature and have been used to evaluate aspects of Shiga toxin-producing E. coli infections. These models may have potential for evaluation of therapeutic intervention to treat these infections.

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There are a number of features of Shiga toxin-producing infections that we would like to see replicated in a nonclinical model. Ideally, after oral exposure to STEC, we should see development of diarrhea and ideally hemorrhagic

colitis. A subset of these animals, or all, should develop some sort of neurological sequelae, and the ideal model would also develop hemolytic uremic syndrome, or HUS, in a manner similar to what is seen in human infants, including thrombocytopenia, hemolytic anemia and thrombotic microangiopathy, particularly in the glomerulus of the kidney.

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As has been mentioned earlier, the hallmark lesion of HUS is thrombotic microangiopathy and it is notable in the glomerulus in the kidney. This particular slide is taken from a pediatric patient in the acute phase of the disease. It shows thrombosis in the glomerulus itself.

DR. RELLER: Dr. Nostrandt, if you could please speak as closely as you can to the mike, we are trying to max out the amplification so that everyone can hear your very important presentation.

DR. NOSTRANDT: Sorry. So, this shows thrombosis in the glomerulus itself and also in the pre-afferent arterial to the glomerulus. Similar

vascular lesions are seen in other target tissues. These also will result in vascular endothelial damage and edema and hemorrhage. Ideally, we should see similar pathological lesions in the animal species.

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I would like to touch briefly on the reported receptor for Shiga toxin. Gb3 is a membrane glycolipid that is present in the membranes of target tissues, and the tissue distribution of this receptor determines the target tissues in the various species that are used in the laboratory.

There is also a differential localization in humans in pediatric renal tissue versus adult renal tissue that has been documented. This may account for the age distribution of HUS, or the age-related incidence of HUS. Gb3 tends to predominate in the glomerulus in the infant in the vascular endothelium. In the adult it tends to predominate in the renal cortical tubules.

After Gb3 binds to the sugar toxin the

toxin receptor complex is internalized and then trafficked within the cell. There are factors that influence the cytopathology that results. It has been shown that sensitive cells tend to have Gb3 with shorter fatty A cell side chains and in those cells that toxin receptor complex is trafficked towards the nucleus or the endoplasm reticulum where it can affect the synthetic capabilities of the cell.

Also, it has been shown that Gb3 is present on human macrophages and monocytes and that it binds Shiga toxin in response to that with production of cytokines. This does not always happen in all the animal models and this interaction in humans may account for some of the differences that we see between the animal models and the human situation.

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A number of different endpoints have been examined in published animal studies. These include mortality; intestinal signs and lesions such as the diarrhea and the typical attaching

interfacing lesions in the colon; neurological signs and lesions. Again, the lesions tend to be vascular in origin, targeted at the vascular endothelial cells. Also, renal endpoints have been examined in a number of species, particularly in attempt to model HUS in animal species. There has been some work done in vitro that looks at the production of cytokines and inflammatory mediators in response to Shiga toxin.

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There have been quite a few different animals used in science. In particular the mouse and the rabbit have been used extensively, most likely due to the low expense and ease of handling of these species. These studies generally tend to be a little bit narrow in scope and have some limitations. There have also been some attempts to develop models in the rat, the ferret, the dog and the monkey. These too have had some limitations and I will talk about them briefly later. Some of the more sophisticated models exhibit the entire spectrum of disease, and these include the piglet

and the baboon. It is very typical in drug development that the initial screening studies are done in some of the more inexpensive species that can be used in greater numbers and then, as better models are identified, these tend to be the ones that are used for proof of concept studies.

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The first model I would like to go into a little more detail on is the mouse. There have been some difficulties cited in the scientific literature in establishing gut colonization with STEC disease. Often what some of the laboratories will do is either pretreat the animals with streptomycin to sterilize the gut prior to inoculating with STEC, or there has been some success with taking enteric strains of E. coli, transfecting them with genes for Shiga toxin and then using them to colonize the gut. This seems to be somewhat strain specific for the E. coli.

The lesions found in the kidney following either oral inoculation or intravenous Shiga toxin administration include acute renal tubular

necrosis. This occurs in the mouse in a subset of medullary and cortical tubules and is probably reflective of differences in Gb3 receptor distribution from what is seen in the human.

In mice there is no effect on the vascular endothelium of the glomerulus so they don=t exhibit the hallmark lesion of HUS. Mice also tend to not develop diarrhea or intestinal lesions consistent with what is seen in humans. They do develop central nervous system lesions and central nervous system signs, and these have been evaluated in some laboratories and these do seem to be resulting from targeted effects on vascular endothelium. However, the primary endpoint that has been evaluated in the mouse model has been mortality.

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There have been several laboratories that have shown modulation of effect by endotoxin. Barrett and coworkers, in >89, showed in the mouse and also in the rabbit that co-administration of endotoxin could either enhance toxicity from Shiga toxin or it could actually protect, depending on

the timing of administration relative to Shiga toxin.

A recent study was published, in December of last year, by Keepers and co-workers. In that model they administered Shiga toxin and endotoxin intraparitoneally. They did see signs develop in these mice that were more typical of what we see in humans with HUS. However, again, these animals did not develop any diarrhea. They do not mention any central nervous system signs. And, the IP administration of these substances bypasses some steps in the pathophysiology of the disease.

Tesh and co-workers, in 1994, did an in vitro study where they looked at peritoneal macrophages derived from mice. They were able to show that those macrophages have a lower level of receptor for Shiga toxin and that when they bind Shiga toxin they do not respond with the same cytokine production that is seen with human monocytes and macrophages. So, the differences in development of disease may in part have some relationship to this difference in cytokine

response.

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In summary, studies that have been done using the mouse model have used primarily adult animals. They have identified renal lesions in these animals but they are usually located in the tubules of the kidney and not in the glomerulus. They don=t exhibit the characteristic thrombotic angiopathy in the glomerulus that is seen in HUS. Mice to not tend to develop hemorrhagic diarrhea. They do develop central nervous system lesions but their immune system does not respond to Shiga toxin with the same cytokine production that is seen in humans, and mortality has been the main endpoint that is examined. These animals seem to have some utility as a screening model, but the differences in pathophysiology and response to Shiga toxin and the expression of disease relative to humans make it difficult to extrapolate these findings to human patients.

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A number of studies have also been

conducted in the rabbit. These animals have been administered orally either Shiga toxin or STEC strains. They do tend to develop diarrhea and they develop typical attaching interfacing lesions in the cecum. Renal effects seem to be species specific. Laboratories that have worked New Zealand rabbits using white rabbits, tend to report that after oral inoculation they don=t see typical renal lesions. However, there has been at least one laboratory that has seen renal lesions in the rabbit that are characteristic of the glomerular lesions seen in HUS after both natural and experimental infections. These are in Dutch Belted rabbits as opposed to New Zealand whites.

There have been other laboratories that have administered Shiga toxin intravenously. These labs have also identified intestinal lesions, as well as central nervous system lesions that coincide with the localization of Gb3 receptors in the brain by immunohistochemical methods. Some of these studies have described renal lesions in the rabbit but have described them as being different

from what is seen in humans. As I mentioned earlier, there has been some work that shows that there is modulation by endotoxin in these animals.

The rabbit seems to be a preferred species for looking at central nervous system effects so some laboratories have injected Shiga toxin intrathecally and looked at central nervous system effects exclusively.

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In summary, the rabbit does tend to develop diarrhea after exposure to Shiga toxin or Shiga toxin-producing species. It is not always hemorrhagic. However, the lesions that are seen in the cecum are typical of thrombotic microangiopathy and they do exhibit some hemorrhage in the tissue itself.

These animals develop classic CNS lesions and this has been the main endpoint examined in the species. Some studies have reported characteristic renal lesions, specifically in Dutch Belted rabbits. There is some information available from experiments that have been performed in juvenile or

neonatal animals, which would be more similar to the patient population in humans.

One of the major drawbacks to the rabbit is that they are exquisitely sensitive to disruption in the balance of gut microflora. I mentioned earlier that the lesions tend to occur in the cecum in the rabbit. The cecum makes up a large portion of the hind gut in the rabbit. They tend to be cecal digesters. This is where a great deal of bacterial fermentation takes place of ingesta. Unfortunately, with the rabbit, particularly with antimicrobial administration by the oral route, when the bacteria get disrupted they can suffer severe consequences and this could confound any kind of investigation of therapeutic intervention.

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The next species I would like to mention is the dog. Hertzke et al., in 1995, identified an idiopathic cutaneous and renal glomerular vasculopathy in racing greyhounds. These animals presented with cutaneous

ulcers on the distal extremities. They also presented with thrombocytopenia and acute renal failure. On histopathology the renal lesions were similar to those seen in HUS in humans. There does not appear to be any data describing Gb3 receptor location and density in dog tissues, however.

There have been some laboratories that have attempted to develop a model of HUS in the species. Raife and co-workers, in 2004, published a study where they described a model of HUS in the greyhound after parenteral injection of Shiga toxin-1 and Shiga toxin-2. These animals did develop bloody diarrhea and the classic renal lesions. They used this model briefly in a small pilot experiment to look at therapeutic intervention aimed at procoagulant changes but there has not been extensive use of the dog as a model for HUS at this time.

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There have been some attempts to establish models in a few other species. The rat has been used. One laboratory has described injecting these

animals with ricin and endotoxin and have shown those animals develop a renal lesion that is similar to that seen in HUS.

Another laboratory has taken rates and perfused kidneys unilaterally in these animals, via the renal artery, with Shiga toxin but the lesions that they see after that procedure have been tubular and not glomerular.

Another laboratory tried to develop a model in the ferret. These animals were pretreated with streptomycin to sterilize the gut and then orally administered STEC species. They don=t see much in the way of diarrhea in this species and they don=t see the typical attaching interfacing lesions in the intestine. However, they did see in about a quarter of the animals at least one sign that was consistent with HUS, such as thrombocytopenia or hematuria.

Finally, another laboratory attempted to develop a model in the Bonnet macaque. These animals were administered 0157 orally. Most of the animals did develop a watery diarrhea and

histopathologically they did have attaching interfacing lesions in the colon as well as intestinal mucosal disruption. However, in the kidney there were no glomerular changes and there was only moderate vacuolization shown in kidney tubules.

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The next species that has been used has been the baboon. These animals have been used as a model of injection of Shiga toxin-1 or 2 intravenously. They have seen effects of dose, both magnitude of dose and the pattern of administration, the dosing regimen, on the toxic effect. But the toxic effects seen in baboons in response to Shiga toxin include thrombocytopenia, hemolytic anemia and azotemia. These animals don=t tend to develop frank diarrhea but they have been reported to exhibit melena. Pathologically, they do exhibit glomerular thrombotic microangiopathy in the kidney, as well as proximal tubule epithelial necrosis. They have also exhibited intestinal mucosal epithelial necrosis.

There has been no mention of central nervous system findings in this species in the published reports, and at least one report has stated that the brains in these animals were histologically normal. One of the major drawbacks to the baboon is that they are expensive and very difficult to obtain. They are not readily available. They are difficult to use and require a lot of special equipment. So, they are used in relatively few laboratories.

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The last model I would like to discuss is the piglet. Pigs are susceptible to natural and experimental disease due to STEC species. Gunzer and co-workers, in 2002, described their model in gnotobiotic piglets. These animals were cesarean derived and placed in sterilized isolators so they had a known bacterial flora composition to the gut. They were inoculated orally with STEC species, including 0157. These animals developed watery diarrhea, locomotor disorders and also several animals had increased serum creatinine. When they

were examined pathologically, the kidneys showed the typical thrombotic microangiopathy in the glomerulus. They also were able to identify fragmented red blood cells in blood vessels in the kidney.

Intestinal pathology included attaching interfacing lesions in the colon, as well as erosions and petechia in the mesentery. As I mentioned, these animals developed CNS signs. They also showed pathological signs of edema and focal microhemorrhages in the brain and spinal cord. This study also did some immunohistochemical staining for Gb3 receptors in the kidney and they were shown to stain in the pre-glomerular arterials. This site was consistent with the sites of binding for Shiga toxin-2 in these tissues.

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These are two photomicrographs from animals in the study. Here you can see a fibrin thrombus in a pre-glomerular arterial. In the second panel, in the upper left-hand corner is a pre-glomerular arterial that is partially occluded

by swollen and proliferating endothelial cells that have also separated from the basement membrane of the arterial. In contrast, at the lower part of the photo you can see a normal artery that is still relatively normal.

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Dean Nystrom and co-workers have authored several publications, primarily looking at central nervous effects in piglets. They have inoculated orally 0157 and other STEC strains into piglets including both cesarean derived colostrum-deprived animals and also two naturally farrowed sucking piglets. They have seen typical intestinal and CNS signs and lesions. Intestinal signs include diarrhea and lesions in the colon and cecal mucosa, including attaching interfacing lesions.

Central nervous signs have included tremors, paresis, high limb paralysis and convulsions. Pathological examination has demonstrated vascular CNS lesions and cerebellum and spinal cord meninges. These have included hemorrhages and edema. They did note in their

publications that vascular lesions were seen in the kidney but, since the focus of their publications was the CNS, they did not describe those lesions in detail.

Winter and co-workers in this 2004 publication was documenting immunohistochemical staining and binding for Shiga toxin-1 and 2 and also for Gb3. They did demonstrate Shiga toxin-1 and 2 binding in target tissues in piglets. They also demonstrated immunohistochemical localization of Gb3 in alveolar macrophages in piglets and peripheral white blood cells from adult pigs. These also exhibited binding of Shiga toxin. They noted that the binding to polymorphonuclear leukocytes and to monocytes was consistent with what is seen in humans.

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Finally, Pohlenz et al. did a retrospective study of renal tissues from multiple studies of Shiga toxin in piglets. These animals were all orally infected and this included both cesarean derived and colostrum-deprived animals and

naturally farrowed suckling piglets. They found characteristic renal lesions of HUS, including the glomerular thrombotic microangiopathy in 82 out of 122 inoculated piglets but in none of the controls. They also did immunohistochemical staining for Gb3 receptors and they found that the renal lesions were consistent with sites that also stain for these receptors.

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This is a photomicrograph from their study. As you can see, there are thrombotic lesions in glomerular capillaries that have occluded the capillaries.

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Here is another thrombotic microangiopathic lesion in a capillary. This not only shows the fibrin thrombus in the lumen but also detaching endothelial cells.

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In summary, the piglet model has used juvenile or neonatal animals, as is consistent with the patient population. They tend to be

anatomically and physiologically fairly similar to humans. After oral inoculation of STEC species we see a similar pathophysiology and spectrum of effects as what is seen in humans. The include colitis and diarrhea, central nervous signs and also signs consistent with HUS, including thrombocytopenia, fragmented erythrocytes and glomerular thrombotic microangiopathy.

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In conclusion, there have been quite a few animal species that have been used to investigate effects of STEC or Shiga toxin in animals. Some of these species have been used to investigate specific narrow focus of effects, while others have been used to characterize a wider spectrum of findings. Depending on the species, they are variable in their similarity to human disease.

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Ideally, an animal model should exhibit disease that is similar that is seen in human patients. It should be as representative of the age and physiological status of the patient

population as possible. The model should be repeatable and validated, and it should lend itself to the investigation of therapeutic modalities for one or more aspects of the disease. While there is no perfect model, I believe that the piglet best fits these criteria. Thank you.

DR. RELLER: Are there any questions for Dr. Nostrandt? Dr. Edwards?

DR. EDWARDS: While the Gb3 receptors vary in the adult and the child human, it would seem logical to look to see if there is a different distribution in the infant mouse model. You mentioned that there has been almost nothing done in the infant mouse. Is there any information that would indicate there is a different distribution in the infant mouse model?

DR. NOSTRANDT: I haven=t seen anything to that effect. There may just be some difficulty dealing with mice just because infant mice are so very small that it would be difficult to try to treat them or dose them accurately. I mean, histopathologically, obviously, yes, those kidneys

could be examined and stained immunohistochemically I would think. But they are really small and probably difficult to work with this model.

DR. EDWARDS: They are more difficult but they have been used for a number of different studies. I was wondering, you mentioned that in the piglet the location of the lesion corresponds to the location of the receptors. I was wondering if the location of the receptors is virtually identical to the location in the human in the kidney. Do you see what I mean? There is a little bit of a difference there.

DR. NOSTRANDT: It tends to be in the kidney cortex and the vascular endothelium of the glomerulus. I guess it is as specific as that has been located, it seems to correlate, yes.

DR. RELLER: Dr. Ward?

DR. WARD: Could I continue about the issues about the Gb3 receptor? You mentioned that there are variations in chain length for the side chains. Within one individual are they constant?

DR. NOSTRANDT: I believe in target tissues

they tend to be constant. So, in a target tissue that is normally where you see true toxin effects they tend to be the shorter fatty A-cell side chains. They are usually 16 or 18 carbons long. But it is in the non-target tissues where the longer side chains are seen, and those toxin receptor complexes tend to be trafficked more towards endocells where they are destroyed rather than producing overt toxicity.

DR. WARD: Do we know anything about in humans whether variation in chain length might influence their susceptibility to progression of disease or severity of disease?

DR. NOSTRANDT: Not specifically-I don=t know specifically, just that in the target tissues that tend to be affected you have the shorter chains.

DR. RELLER: Thank you very much. Our final speaker before our lunch break will be Dr. Yan Wang, who will present the FDA=s perspective on study design in relation to trials for hemolytic uremic syndrome intervention.

Study Design Issues and Considerations in HUS Trials

DR. WANG: We are a little bit late for lunch but I hope you are not too hungry to listen to my talk.

DR. RELLER: Again, please speak as forcefully as you can directly into the microphone so everyone can hear your presentation. Thank you.

DR. WANG: My presentation today is about study design issues and considerations in HUS trials.

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I will first briefly talk about the key element of adequate and well-controlled studies. Then I will go through some study design issues and HUS trials. In particular, I will focus on issues related to choice of primary efficacy endpoint and its impact on the efficacy evaluation and sample size requirement. I will also discuss sample size considerations for the safety evaluation. I will conclude my talk with a restatement of the challenges involved in the design of these trials.

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As mentioned in Thomas Smith=s talk, for approval of a new product data from adequate and well-controlled studies are needed to demonstrate effectiveness and safety of the product. What constitutes an adequate and well-controlled study? According to 21 CFR 314.126(b), an adequate and well-controlled study should have the following characteristics, number one, there is a clear statement of the study objectives. Number two, the study design permits valid comparison with appropriate control to provide quantitative assessment of drug effect. Number three, the study selects patients with disease or at risk of disease. Number four, a proper method, such as randomization, is used for treatment allocation to assure comparability of the study groups. Number five, adequate measures, such as blinding and randomization, are taken to minimize bias. Number six, there are appropriate methods to assess the efficacy and safety outcomes. Finally, appropriate analytic methods are used to assess the effects of

the product.

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We expect that HUS prevention trials are adequate and well-controlled and prefer that they have the following characteristics: The trials are randomized, double-blind with a placebo as a control group. The target population may include patients with STEC infection and at the risk of developing HUS. The incidence of HUS is used as the primary efficacy endpoint to measure the treatment effect on HUS prevention. The trials are sized to have adequate statistical power to detect a treatment effect. The trials should have adequate data to demonstrate safety.

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We face some challenges for design of HUS prevention trials. As mentioned in Dr. Griffin=s talk and Dr. Tarr=s talk, the STEC infections are rare and sporadic events, especially in the United States. This creates logistical issues in selecting study size and getting IRB approval in a timely manner. No prognostic variables have been

identified to predict HUS development from STEC infections. A therapeutic window may be narrow, possibly within 48 hours of the infection. And the incidence rate of HUS in patients with STEC infection is relatively low, ranging from 5 percent to 15 percent. All these issues together make it difficult to find a sufficient number of patients for HUS prevention trials.

[Slide]

In this slide we present a sample size calculation required for efficacy evaluation in a placebo-controlled HUS prevention trial under various scenarios, 3 placebo incidence rates, 5 percent, 10percent and 50 percent are considered. In the treatment effect we tested 33 percent, 50 percent and 75 percent reduction of placebo rate. The required sample size for efficacy highly depends on the placebo incidence rate of HUS. For example, in the middle panel the red bar shows that if the placebo incidence rate of HUS is 10 percent, 435 patients in each group are needed to detect a treatment effect of 50 percent reduction of placebo

rate. If the placebo incidence rate of HUS decreases from 10 percent to 5 percent, the required sample size will be more than doubled. The required sample size for efficacy also highly depends on the treatment effect to be tested. Comparing the numbers in the middle panel with the numbers in the right panel, we can see that the required sample size can be reduced by more than half if the treatment effect increases from a 50 percent reduction to 75 percent reduction of placebo rate.

So, as we can see, by enrolling patients who are more likely to develop HUS or focusing on developing more effective therapy we will reduce the sample size for efficacy evaluation.

[Slide]

Another strategy for reducing sample size for efficacy evaluation is to use a composite endpoint as the primary efficacy measurement. When a composite endpoint is used the number of events increases. This will reduce the sample size required for efficacy. Can we use a composite

endpoint in an HUS trial? This is one of the questions on which we are seeking your advice today.

[Slide]

For demonstration purposes, in this graph we use a hypothetical composite endpoint. We assume that this hypothetical composite endpoint has an incidence rate of 30 percent. Using this hypothetical composite endpoint, the required sample size will greatly reduce compared to the cases when HUS incidence is used as the primary efficacy endpoint, as shown in the previous slides.

Use of a composite endpoint can reduce sample size for efficacy evaluation, but it creates difficulty in interpretation of the results when treatment effect on components are not homogeneous. This graph shows a hypothetical example of an HUS trial using a hypothetical composite endpoint. The composite endpoint comprises 3 components with HUS events as the most important component and the third component as the least important component. The result of the composite endpoint analysis is

positive and statistically significant. However, it is driven by the results of the third component, the least important component. No treatment effect is shown on HUS prevention.

How do we interpret these kind of results where the treatment effect on a composite endpoint cannot be translated to effect on HUS prevention? Is the result of the composite endpoint clinically meaningful? To find a satisfying answer to this question may be very difficult.

[Slide]

To avoid difficult situations like this, and to ensure that an effect on HUS prevention can be inferred from an effect on a composite endpoint we need to have data to address the following issues at a study design stage, are the individual components clinically relevant and of similar importance to patients? Do the more and less important endpoints occur with similar frequency? Is the underlying pathophysiology of the components similar? Are the components likely to have similar relative risk reductions?

[Slide]

So far we have discussed the following issues in HUS trials: The sample size can be prohibitive if prevention of HUS is used for efficacy measurement. Use of a composite endpoint can reduce sample size for testing treatment effect, but it will create difficulty in interpreting the results of the composite endpoint, and the treatment effect on the composite endpoint cannot be translated to an effect on HUS prevention.

[Slide]

Now I would like to talk about safety evaluation. Why is it necessary to have an adequate number of treated patients for safety evaluation? First, let=s look at the chance of observing no serious adverse event. What does it mean when no serious adverse event was observed or no safety issues were identified in a clinical trial of a new product? It doesn=t necessarily mean that the new product is safe because the chance of observing no events can be high when the

trial size is small.

The chance of observing no events depends on the incidence rate of the serious adverse event, p, and the number of patients treated in the trial. It can be calculated using the format in the top of the table. For example, if a serious adverse event occurred with an incidence rate of 1 percent and 100 patients are treated, the chance of observing no event is 37 percent. Another example, if a serious adverse event occurs with an incidence rate of 0.5 percent and 300 patients are treated in a trial, the chance of observing no events is still high, 17 percent.

In the real world we don=t know the true incidence of a serious adverse event for a new product. We conduct clinical trials to estimate it. What can we say about the risk of a product when no event is observed in a clinical trial?

[Slide]

The rule of three provides us a simple answer to this question. The rule of three states that if no events occur in N treated patients, the

upper bound of the 95 percent confidence interval for the risk ca be estimated as 3/N. Rule three provides us an answer to a very important clinical question, what is the worst possible scenario for the risk of a serious adverse event when no events occur in the trial. For example, as demonstrated in this graph, if 100 patients are treated in a trial where no serious adverse event occurs, the risk of a serious adverse event can be as high as 3 percent. Another example, if 300 patients are treated in a trial where no event occurred, the risk of a serious adverse event can be as high as 1 percent.

Rule three can also be used for sample size calculation. In order to rule out the risk of a serious adverse event we do not exhibit a given threshold if there is no event occurring in trials. Again, based on this graph we can see that in order to rule out that the risk of a serious adverse event is no more than 1 percent we need to treat at least 300 patients. In order to rule out that the risk of a serious adverse event is no more

than 0.5 percent we need to treat at least 600 patients. As was discussed in slide number 8, treating less than 300 patients may be sufficient for efficacy evaluation but it may not be adequate for safety evaluation according to the rule of three.

So, the rule of three tells us that it is very important to have adequate data for safety evaluation. The following considerations further suggest that it is particularly important to have adequate data for safety evaluation of a new product for HUS prevention trials.

[Slide]

Because the majority of the patients with STEC infection may not benefit from a prophylactic therapy for HUS, as was mentioned before, the incidence rate of HUS in patients with STEC infection is in the range of 5 percent to 15 percent. In other words, 85 percent to 95 percent of the patients with STEC infection will not develop HUS.

In addition, the number of patients needed

to be treated to prevent one HUS case could be large, as shown in the following table. Example, if the placebo incidence rate of HUS is 5 percent and the treatment effect is a 33 percent reduction, we need to treat 60 patients to prevent one HUS case. In other words, 98 percent of the treated patients may not benefit from the therapy.

Another example, if the placebo incidence rate of HUS is 10 percent and the treatment effect is a 50 percent reduction of the placebo rate we need to treat 20 patients to prevent one HUS case. In other words, 95 percent of the treated patients may not benefit from the therapy.

In the most favorable case when the incidence rate of HUS is 15 percent and the treatment effect is a 75 percent reduction of the placebo rate we need to treat 9 patients to prevent one HUS case. This means 89 percent of the patients may not benefit from the therapy.

[Slide]

In conclusion, we need to have adequate and well-controlled clinical trials to evaluate

efficacy and safety of new products for HUS prevention. There are many daunting challenges for designing HUS prevention trials with adequate statistical power to evaluate a clinically meaningful efficacy endpoint and with sufficient data to demonstrate safety. Can we overcome these challenges to design such trials? We are seeking your help today to address these issues. Thank you.

DR. RELLER: Dr. Moxey-Mims?

DR. MOXEY-MIMS: I am not sure if you are the right person I should address this to. It is a policy or process question and I don=t know, maybe Dr. Smith would be the one to answer it. But based on the statistics that you have just shown us and the presentations by Drs. Griffin and Tarr earlier about the very low incidence in the U.S., and we know that there is a significantly higher incidence of the disease in Argentina, for example, is there a policy issue that would prevent the primary study being done, say, in Argentina where you have a much higher incidence? I don=t know if the assumptions

are correct that there would be, say, no difference in the kids there versus here except for their exposure to the risk. Is there anything else inherently different from those children that would prevent the data from being extrapolated to kids in the U.S.? And, if the conversion rate from an STEC infection to full-blown HUS is the same, then certainly with the higher number of people with STEC infection it would seem it would be easier to accumulate the amount of data that you need. So, if that were done, what is the FDA=s policy on what would then be required to bring a product, shown effective in that situation, to market here in the U.S.?

DR. WANG: That is a big question.

DR. RELLER: Dr. Soreth will answer this question for us.

DR. SORETH: The regs. allow for inclusion of foreign data in a product development program. So, as a matter of policy, there is no problem with the inclusion of foreign data and in the development of a product, for example, for malaria

the overwhelming majority of data, if not all the data, come from ex-U.S. experience.

In the case of developing therapies for prevention of HUS with STEC infections, I think probably Dr. Tarr and Dr. Griffin would agree it would certainly be nice to include patients who developed this in the U.S. But can a development program go forward solely with U.S. or North American patients? We have seen some of the data already. That is probably not likely to happen and one, I think, would need a global or international development program. Does that answer at least part of your question?

DR. MOXEY-MIMS: Yes. I guess my question still would be if the primary study were done there, what would be your requirements in terms of are there specific numbers or specific percentage of patients that would have to come from U.S. sites?

DR. SORETH: I am not aware of anything in the regs. that calls for a specific number or percentage of cases. You would like to know, given

the information that you have about the U.S. experience, that the findings which would be developed outside the United States be generalizable. So, if there are data to speak to differences in the pathophysiology of the disease, then that would be pertinent. But if the data that we do have at hand for the U.S. experience is such that we think we are dealing with similar infections, similar rates of development of HUS, similar sequelae, and similar immune systems, and so forth, there would be no reason, I would think offhand, to reject the generalizability of a program that was primarily conducted outside the United States because most of the infections are outside the U.S.

DR. MOXEY-MIMS: Thank you.

DR. RELLER: Mis Dokken?

MS. DOKKEN: Is there an FDA definition of a rare disease? If so, are there special considerations in terms of trials? Lastly, does HUS qualify as rare based on the incidence?

DR. RELLER: Dr. Smith?

DR. T. SMITH: We have a definition for conditions that would apply for orphan drug category, and that is less than 200,000 cases in the United States. So, prevention of HUS would fall under that category.

In terms of special treatment, you know, there are some provisions for drugs that get this designation, drugs or biologics that get the designation. But orphan drug status in and of itself doesn=t eliminate the need to perform adequate and well-controlled studies for approval.

DR. RELLER: Dr. Rappley is next.

DR. RAPPLEY: Just to mention that if consideration is given to doing clinical trials in other countries, and recognizing the problems with bringing advanced technology and very expensive medication to some of those countries, that would need to be considered in terms of consent and use of those populations for clinical trials and availability of treatment should it prove to be effective.

DR. RELLER: Dr. Wong-Beringer and then Dr.

Ward.

DR. WONG-BERINGER: I am just reflecting upon the informative talks from this morning, looking at the lack of a precise biomarker to identify patients who may develop HUS, the daunting number of patients that are required to target for a clinical trial to look at effectiveness of a prevention trial, I think also looking at the important role that appears that the Gb3 receptors may plan in the pathogenesis of disease, I wonder if Dr. Tarr and Dr. Griffin can comment on whether there are existing specimens that we could look at, and look at human genetics from that perspective, Gb3 receptors, and whether we can screen patients and see whether there is a relationship to those developing HUS.

DR. RELLER: Dr. Tarr, do you want to respond to that?

DR. TARR: We partly looked at that with the P blood group several years ago and did not find an association, any protective or harmful association with people variably expressing P blood

group antigens. DNA is saved from our cohorts of patients. We have no idea which group to go after. We have no idea which locus to go after. We have looked at some prothrombotic indices, looking for high risk factors and haven=t found any. Gb3 can be a hard thing to find on the target tissue because you don=t have access to the renal glomerulus or endothelium, which would be critical in a human population. Another person in the audience, Dr. Martin Bitzan, might also be able to address some of this.

DR. RELLER: Dr. Bitzan will be presenting later so we will ask him to think about this in relation to his presentation. Dr. Ward?

DR. WARD: Dr. Wang, you described measuring safety in the same cohort as measuring efficacy and that is certainly how we usually do clinical trials. But is there an opportunity to study safety in an adult population that doesn=t have bloody diarrhea or STEC that might help to inform us about safety of this product separate from the efficacy trial, recognizing we would

combine safety with efficacy in the clinical trial but we might get a better understanding of safety in another population?

DR. WANG: That is another good question I think.

DR. RELLER: Dr. Soreth?

DR. SORETH: You raise a good point, and I think that another population could provide some information but the question would remain would it be enough. If this is primarily a problem in the pediatric population, and it appears to be, then I think the best data would be derived from that population, including someone who has the disease of interest, to really know if use of a monoclonal or any other therapy has unique toxicity issues. We wouldn=t necessarily get that in the adult population. We wouldn=t necessarily get it in an uninfected population.

DR. RELLER: Dr. Edwards?

DR. EDWARDS: My question I think is somewhat similar. The rule of three applies to patients with illness and not to normal volunteers. Is that correct?

DR. WANG: I am talking about the rule of three applying to the target population you are treating. We decided to apply this new product to the patients who are at risk of developing HUS, so we are talking about the safety of this target population. It is not the safety of those patients who will develop HUS. It will be a very small portion of the study population. So, this is like a prevention trial in general. You give the medicine to many people. You want to make sure the medicine is safe for the general population.

DR. EDWARDS: I just have one other comment that I need your editorial one. That is, if we assume 435 patients are necessary for a 50 percent signal of efficacy, according to the Klein 3-year study, I calculate it would take 87 years--

[Laughter]

B-to do that study in one center. I think that is correct. Then, if you go down to the composite endpoint it would be approximately 56 years. The magnitude of those years, if one adds foreign sites in, you could imagine you would have

to be adding a lot of additional sites to get that down to a 3- to 5-year study period. It would be a tremendous number I think. Are those correct assumptions?

DR. WANG: That is correct. So, in a sense, it also shows us that if somehow we can refine the study population so that there is higher risk of developing HUS, say 15 percent, we don=t need 435 patients treated. We only need 120-some patients treated for efficacy evaluation. So, that is another way to think about whether we can overcome these daunting numbers.

DR. WARD: And in the spinach outbreak cases it was 15 or 16 percent progression overall, and in the children wasn=t the progression 24-30 percent to HUS, from this morning=s data?

DR. GRIFFIN: Yes, that is correct, but that was an unusually virulent strain.

DR. RELLER: So, we have just heard sequentially about this by Dr. Ward and Dr. Griffin. We will have two more questions before lunch, Dr. Hilton and Dr. Daum, and the most recent

series of questions are the very things that we will be wrestling with this afternoon to look at reality case definition endpoints, composite or otherwise. Dr. Hilton and then Dr. Daum.

DR. HILTON: For the clinicians I have a question. Is HUS a late clinical stage of developing vascular injury that begins with 0157 infection?

DR. TARR: I am sorry, could you repeat that?

DR. HILTON: Is HUS just a late manifestation of developing vascular injury that begins with infection with, say, 0157?

DR. TARR: Almost everybody who has an E. coli infection has vascular injury if one uses sophisticated coagulation studies. The result of that cascade, in our definition renal impairment was the case-maker definition by an elevated creatinine and that is what we are referring to as the late consequence of the enteric infection, the late defined as a week later.

DR. HILTON: If that is so, then it seems

that clearance of 0157 infection could be an endpoint because infection is much more prevalent, so you are talking about composite endpoints to try to get more events. So, this is one way to increase your number of events and try to show efficacy.

DR. TARR: Probably not in this infection because the toxemia, which may have occurred before presentation or at the time of presentation or will continue to occur for several days, is really the target. The organism, if not incidental to it, is not a sufficient surrogate or a composite component. The organism by itself isn=t going to cause renal injury.

DR. RELLER: Dr. Tarr, who was just speaking, is saying and presented in his presentation that persistence of the organism and being able to detect even the toxin in stool-Bit is not there at the time that the end result of those who don=t approach and recede from the brink but, in fact go over the brink with anuric HUS. So, it is not exactly as stepwise as you outlined but an

end complication without evidence of the initiating event at the time of the occurrence. Is that correct, Dr. Tarr?

DR. TARR: I think so, yes.

DR. RELLER: In a summary form. Dr. Daum?

DR. DAUM: My question goes to agency policy really about the safety issue and I would love to hear comment. It seems to me that we have bandied about several different issues with respect to safety in terms mainly of the target population. Someone talked about doing safety assessment in healthy adults and someone else mentioned doing a safety assessment in healthy children. Then, Keith reminds me that there could possibly be a safety assessment in elderly adults because they have a high risk of disease as well.

But it seems to me that none of those issues get at the possible difference in safety that people who actually have the disease might have. So, I guess I am wondering, from an agency perspective, and I pick on Dr. Soreth particularly because I think your remarks went to this issue

more than any other, what would be the perspective on safety assessment in healthy adults and elderly adults and children, and then supposing you had all that but nothing about patients with the disease? I am just trying to work with your numbers, and I find them, obviously, very daunting.

DR. SORETH: Well, I think it is a difficult issue, and I think before we would be comfortable with the overwhelming majority of safety information coming from other populations-Belderly adults, younger adults, uninfected children-Bwe would first think long and hard about other measures, perhaps an open-label follow-on type of experience where you don=t have the same problems as a controlled clinical trial experience but you try to get the numbers that way. Dr. Nelson, did you want to make a comment?

DR. RELLER: That was Dr. Soreth and now Dr. Nelson.

DR. NELSON: The use of healthy children would be off the table. It wouldn=t be in compliance with Subpart D.

DR. SORETH: Thank you, very clear.

DR. RELLER: In preparing for our lunch break, Lt. Mosaddegh has some instructions for us.

LT. MOSADDEGH: Thank you. The advisory panel, if you would like to all stay together, we have reserved 25 seats at the café directly across our building. You will need to go upstairs and walk directly across Fishers Lane to 5635 Fishers Lane. It should take you about 30 seconds. There are three FDA staff members right outside those doors who can walk you in groups so no one gets lost. Feel free to go anywhere else if you like. I will be in this room if you would like to leave anything behind. Thank you.

DR. RELLER: In the interest of having a full discussion of this very complex issue this afternoon, I would like to ask that we reconvene at 1:30. Thank you.

[Whereupon, at 12:45 p.m., the proceedings were recessed for lunch, to reconvene at 1:33 p.m.]

A F T E R N O O N P R O C E E D I N G S

Open Public Hearing

DR. RELLER: We will begin this afternoon's session with our Open Public Hearing. We have one speaker, Jonathan Stern, who will have a three-minute presentation. Again, I remind you, appropriate disclosures, please. Mr. Stern?

DR. STERN: Good afternoon. My name is Jonathan Stern. I represent Inverness Biostar. We are the manufacturers of a recent 510(k)-cleared test for Shiga toxin that I wanted to make the group aware of since people were talking about diagnostics this morning because I think this has a bearing on the rest of your afternoon.

Our test is for all Type 1 and Type 2 Shiga toxins. It is a stool test. It requires only 15 minutes from direct fresh stool and has a sensitivity--percent agreement, excuse me, I will phrase it properly--100 percent positive agreement and 98.1 percent negative agreement from direct stool.

It can also be run from broth culture with