Dr. Legendre: Certainly I want to start off by thanking Steve. Can you hear me back there?

Male Speaker 2: Yes, go ahead.

Dr. Legendre: Okay. I certainly want to thank Steve for this wonderful exaggerated introduction, and also thank the Winn Foundation for the invitation to come in and present to this group the work that is supported by the Winn Foundation. In addition, I certainly am proud and humble to be on the same stage with Niels Pedersen that I consider the "father of feline infectious peritonitis research" who has been working in that area and has made so many contributions. We want to go ahead, and let us look at the polyprenyl immunostimulant which is the product that we have been working with as it relates to feline infectious peritonitis and to talk about the polyprenyl itself. I want to go ahead and start looking at this complex origins of this product and to go ahead, we see on the Russian bear here, we will see Arthur Sas and we will see Tanya Kurtz. Arthur and Tanya where are you seated? Back over there. They are the ones that developed this product. This comes out originally out of a group of products that we looked at starting way back in the era of the Soviet Union and then in Russia, developing a group of these products, going ahead in conjunction with a variety of different groups. Which Winn Foundation is certainly good-hearted people who looked at the analysis of the drug, people who have done safety studies.

Back in 2003, the US Department of Agriculture agreed that they will be the regulators of this product which is up for licensing, so that we're hopeful in a very short period of time that this product will be licensed and will be available to veterinarians to use as an adjunct to other treatments. Looking at the Oak Ridge National Laboratory that we are involved, we have two institutes, Veterinarian Institute and the Poliomyelitis Institute in Moscow. Now, both Arthur and Tanya are from Moscow. Originally, Tanya has a PhD in molecular biology and Arthur also has a PhD and did a lot of the work for the statistics on this project. So that is some of the background information that we have on the origin of the polyprenl and here is a picture that Tanya put together. We were involved with the clinical studies within that one with rhinotrachietis because there is certainly another significant problem in cats as well as the FIP and doing some of the safety studies. I am sure this is fascinating to everyone. There is some in the area of organic chemistry that might be of interest as to the structure of the polyprenyl immunostimulant. One of the things that we addressed with our safety studies is to meet the USDA safety studies. We are very pleased to get products through the USDA that handles biologics because it's so much easier to deal with those people than it is to deal with people that at the FDA, but they still have a lot of hurdles to jump.

First step was to do no harm, and there was safety studies done with a total of 390 cats in 10 states, the rule say that you have to have three different locations and we wound up with 10 different locations, looking at the dosing schedule that we use for the rhinotracheitis treatment which is what the product is going to be licensed under for early rhinotracheitis and we found essentially no adverse effect, there seems to be a safe product, in the 390 cats, there were three of them that really didn't like the taste of it, one developed diarrhea, and then we have a balance here where two of them, two or three that didn't like the taste. We have looked at this product at 10 times the dose that we are dealing with rhinotracheitis without adverse effect and we have

gone to 100 times that in some mouse studies without any adverse effect, so it does appear to be quite a safe type product. Those of you who are into the immunology side, we still think that this falls in to an agent that stimulates toll-like receptors which tends to direct an immune response to a TH1 type of immune response.

This is a cell-mediated immunity which is what you need for viral infections. So directing, shifting the immune response to a TH1 response through this toll-like receptor mechanism. Our first study that we looked at was looking at feline rhinotracheitis and I have some studies which suggests that they tend to work well against rhinotracheitis and we see looking at some kittens, 6 - 10 weeks of age and 10 that were in the placebo room, and 10 that were on the treatment group and they were challenged where they were given orally and intranasally, a dose of a rhinotracheitis virus, and then there were those of this dose for a period of 14 days or 15 days and we looked at the before and after evaluated clinical signs and this was one of the kittens that was used in the study. His name was Felix and he was the virus. It's interesting we dug those up because we didn't have any baby pictures at home. So what we have in our scheme was we looked at weight loss over the period of time assigning mild; less than 5% of body weight loss or more severe, greater than 5% of body weight loss, one got a 1, one got a 2. The higher the number, the more clinical signs were seen. We looked at the nasal discharge on these kittens. They had moderate light nasal discharge or really thick with a lot of discharge. We looked at whether the nose obstructed with the nasal discharge or not, whether there was salivation, some of these cats can get ulcers in the mouth, not as bad as we can see occasionally. We looked at conjunctivitis or inflammation of the eyes whether again; like in the nasal discharge, whether it's a mild light discharge or heavy discharge, and we looked at moderate hydration and your very typical clinical signs that the kittens with rhinotracheitis will show.

And this is what we saw and as I mentioned, the higher the score, the more clinical signs were seen. Here again, looking at the daily score and looking at their cumulative scores over a longer period of time and you can see on the red line of the placebo group, you know, all cats showed clinical signs of the ones given the placebo had more severe clinical signs than we had on the other ones that received the polyprenyl and you go ahead and look at the two curves; you can see that the red curve, the control curve, at the height, the curve was much higher as it is typically for a lot of these research projects; we were trying to look at statistical difference at the $P = 0.05$ level. We had it at the P.06 level, so now we have got another set of 20 cats that we will be doing to see if we can show that in a larger group of cats, significant differences as an aid to the treatment of feline rhinotracheitis. If we look at the data in a somewhat different way as to how long the clinical signs persisted in our cats. And we're seeing that in this one, our treatment worked here in blue again, in the control group in red, where in the treatment group the signs were pretty much restricted to the first week, so that we have, it showed up about the same time over here, but some of the cats in this group only have about three days and have significant clinical signs, others-four days, six days, seven days, they are pretty much the ones on the experimental group had cleared the infection by the end of seven days, while there seemed to be in placebo group, went on and had problems for much longer, so it does seem to not fully reduce the severity of the rhinotracheitis, it tends to reduce the duration of the rhinotracheitis. So it's not a cure-all, but it does help in controlling this very difficult disease process. Now, looking at feline infectious peritonitis, I don't have to tell a group like this that it's a major problem in multiple cat households, the cats coming from shelter environment, certainly appear to be at

increased risk for developing feline infectious peritonitis. Certainly believe that stress is a complicating feature when we're dealing with the FIP problem and that I am sure Niels is going to be talking further on genetic predisposition, these cats develop FIP. Now, people say what is the world is going on here, this is a study done by Julie Levy at the University of Florida where she is now involved very much in shelter programs, and this is part of the trap, neuter and release program that was there in feral cats.

And in working with the feral cat population, what was noted when I did the antibody titers for corona virus, they use feral cats. And a number of feral cats that carry antibodies to corona virus which is the positive agent of FIP is quite low, so that cats in an environment that are spread out, that don't have that close contact, we know this one is difficult at a dinner meeting, to start talking about fecal-oral transmission of disease. We know that in these feral cats, they go out there, they have many acres to run on, they bury their stool, and then other cats might have come to contact with it. The transmission of the corona virus is not a big feature. But when you're going in and put cats in a crowded environment, you have the stress of having many cats competing with each other, then that's certainly sets up the right situation for the development of the persistent corona virus infection and subsequently FIP.

And we see a variety of things that we find the FIP, certainly interference, a depletion, a suppression of the immune response that is necessary for clearing out the corona virus, and clearing this virus and infection from the body and we know that there is a compensatory overproduction of antibodies. And these are ineffective antibodies so that cats with FIP usually have very high antibody titers, very high body immune modulation that occurs in a situation when you have this persistent antibody production. We felt that the immune-modulator, like polyprenyl immunostimulant that was originally some of these products were originally tested in the Soviet Union in mice that have viral encephalitis, and they have model systems where 100% of the mice died from the viral encephalitis, and when they added polyprenyl to the mixture they started getting survivors in this uniformly lethal infection where uniformly lethal is what's been recognized as what's going on with FIP. So that we figured that FIP would be a good animal model in relation to try this stuff.

Then we went ahead and some of the things we found early in our trials with the polyprenyl immunostimulant, one we found out that with cats that have the effusive or liquid form of FIP we have no success in those animals, we found that these cats seemed to be so far advanced in the course of their disease, that there was very little hope to turning them around. Now in looking at the dry form of the FIP we did see some we felt lived longer than expected, and that's where this is kind of shaky is that how long do you expect them to live. But after looking at some of our poster children in the treatment with the polyprenyl immunostimulant…

We went ahead, this thing (remote) has a hair trigger, we could have had this presentation in 10 minutes. We reported on three long term survivors, and here we have Gringo which is one of the original group, Gringo had intestinal granulomas, the granulomas were biopsied by the original veterinarian they did ilium staining, and showed that there's corona virus within the granulomas. So that was as good as diagnostic choice we could get as hard as determining that the cat truly had FIP and we found Gringo is alive and well, five years now after starting on the polyprenyl immunostimulant. So that was the encouragement, we submitted to the Winn Foundation to go

ahead, and say, is this anecdotal situation just a fluke, or is there a truly any benefit to going ahead and looking at the polyprenyl immunostimulant, we did, we looked at this in the effusive form, soft, no responses, so we focused in only the dry form.

Now, the next step we want to look at is diagnosis, because that was a very key aspect of the last proposal, because with the disease that's uniformly fatal, if you have a cat that lives a long period of time, what's the first thing that skeptics are going to say? Wow, if it lived, it didn't have FIP, it was supposed to die. So you really get caught as to have a credible diagnostic criteria for what you call FIP and as you all have found out who have had cats with FIP, making that call.

You really want to put a veterinarian on the spot, just ask him, can you be a 100% sure that this cat has FIP. Oh hell, I can't be a 100% sure about anything right now, but that is a very difficult aspect, is that knowing how solid your diagnosis is. If you get one like this, that has a young kitten from the shelter that has a belly full of the right kind of fluid. That's a no-brainer for going ahead and calling that FIP. Now, when you're looking at the dry form of FIP what are you looking to make a diagnosis? One, you're looking for evidence of these granulomas that this one is a granuloma current in the liver, and if you find within the granuloma, if your immuno stain that shown corona virus in there, that's probably assuming that there are all the other clinical signs are there, that's as good as it gets in making a diagnosis.

So once in its dry form, we know that it's difficult to make a diagnosis, we go ahead and have our criteria which we'll talk about in a minute. And then here's a kidney, and you can see that one of the characteristics of these granulomas is that they do tend to go along vasculature over here, and you can see on the surface of the kidneys the vessels over here and you can see the granulomas of FIP follow along the vessels, you get this picture of the liver, this granuloma and you see the little blood vessel in the center of it. Now, one of the things that we struggled with in putting the grant together is that being in still preliminary study, we did not have a control group, which is a very, you know, a control group that have been very helpful, but then you go ahead and you don't have an alternate, it's one thing when you have two treatments and you're trying to go ahead and say, which is the better treatment, when you have no alternate treatment you go ahead and say, hey, some of you are going to be in a control group, and your cats are going to die. That creates a good scientific model to have a placebo group, but the real concern from personal, ethical aspects, those come a lot. The other thing that we're looking at in these FIP cases is that we have a situation where a lot of times, is in the long-term making the diagnosis, a lot of times, the disease is quite advanced, by the time that a diagnosis was made, there are very few cats that are diagnosed for FIP, that had not had a course of antibiotic therapy. Well, maybe this is a hepatic caused infection, so the diagnosis of FIP has usually not come early.

So that's another complicating factor. So our criteria for calling it a definitive diagnosis and one of the big ones are the history and physical findings, compatible with FIP. Such as fever, unresponsive to antibiotics, the lack of response to treatment, a progressive type of a disease process. Another major factor is an exclusion of other diseases. We need to have the veterinarians and this was done with veterinarians throughout the country, we did not restrict this to the University of Tennessee. We had cases that were put on the protocol throughout the country, and when they had working with those veterinarians, as to those things that we needed

as part of the workup. One of the big parts is to go ahead and say, you know, have you considered this, and have you consider that? A lot of these animals were essentially, all of these animals were tested with feline leukemia virus, and they were tested for feline immunodeficiency virus. A lot of them were tested for toxoplasmosis, they settled in the right area, they had tests looking for fungal diseases, anything that might mimic FIP. We wanted to see being that the globulins are increased in most of these cats with FIP. We put that in as a criteria, we wanted to see highest globulins or antibody levels, low albumins and the albumin globulin ratio was down. We wanted to see cats that have a moderate to high corona virus antibody titer. We wanted to see that this was truly when we have lesions that were identifiable, that these were truly granulomatous or highly granulomatous changes that we see. And that they went ahead and had a biopsy, or an aspirate of these lesions, if highly granulomatous, and if we could get looking at immuno-staining for corona virus.

And the other things that we had depending on what they came in for, we found that looking at PCR of the fluid in the eyes, these are the ones that came in to ophthalmologist, we get ocular fluids that they found the corona virus was in the ocular fluid. The same thing with finding the corona virus in cerebrospinal fluids, we had some that had MRIs of the brain and I am told by the radiologist that there are some characteristic features of FIP involving the central nervous system, and then going ahead and necropsy findings on those that died, and to date, we have necropsies that results back, on about 8 of the ones that died and that on all of them, there was one with the pathologist was a bit wish-washy as far as calling it. But they didn't call it anything else. This is so typical on pathology reports on cats with FIP and most consistent with feline infectious peritonitis. They never stated there that your cat has FIP consistent with typical some other terminology, so we felt that our criteria was reasonably good to guarantee that most of these cats had feline infectious peritonitis. Now, we also had a situation where we included cats that we had in the probable FIP. And they landed in that area where the people would say, hey I want to participate in this, but I am not going to go ahead and allow a biopsy, to go in and do the exploratory, do a biopsy, a lot of those and of course we have a situation where we did not have a lot of money to work with a diagnostic test. So that the diagnostic tests were paid for by the owners in this case, so that limited what we could go ahead and do. But we had a lot of the same criteria for the probable ones, that history and physical findings compatible with, they excluded other diseases, they have the globulins, they have the high corona virus, antibody titers, they have variety of other things compatible with FIP, where they did not have a cytology or histopathology that supported the diagnosis. Now, looking at the antibody test, as you well know, the antibody tests aren't an end all and be all in there, but it is an indicator and the diagnosis of FIP is a cumulative diagnosis, you have some of this, and some of that, some of that, some of that, and if you have enough evidence that tips the scale to say yes like the pathologist this is probably FIP and we know this work has been done by Sparks, where he looked a group of 28 cats that had FIP and went ahead and look at a 196 cats that had other diseases that didn't have FIP. And we know that the corona virus antibody titers are nonspecific, but we also know that in the ones that had FIP, 96% in his study had some antibodies to corona virus, but there are 86% of those that didn't have FIP. So quite common in the population of cats.

When you start looking at high antibody titers, there's certainly really high values, 25% of the cats with FIP had high values although 6% of the non-FIP cats. So it's a bit of a discriminator but it is not the end-all be-all in this situation. Now, like I said this was a nationwide study, with

a few strong cases that we're able to do with Canada and then we have the permission of the receptive donors send out some polyprenyl to people in variety of other countries. FIP is certainly not unique to North America type situation, had calls from Australia, Singapore, Korea, just from all over the world. We went ahead and there's a variety in here to be used in the diagnostic procedures done by the veterinarians what their usual procedures were and certainly there's a lot of weight, how strongly a lot of the veterinarians felt if it was FIP and the veterinarians that we work with on this were specialists, they were veterinarians at the feline only clinics, a variety for instance the people that really knew what they were doing. We initially had put in to do 40 cats and we wanted to put 40 in, you probably have at least 20 that are generally gold or evaluatable or had good follow ups. We ended up taking in a 102 cats in to the study, and stopped about 6 or 8 weeks ago, to go ahead and start analyzing the data and it was the owners that provided funding for the diagnostic tests or the diagnostic procedures. We have no restrictions on concurrent treatment, all of these cats were on antibiotics, they were on antidiarrheals, they were on steroids, and they were on a variety of other things.

But we did as we could try to decrease the dose of steroids, wean them off of the steroids, because it's certainly, is that, intuitive to say you don't want to use an immunosuppressive drug at the same time that you use an immunostimulating drug. And then we went ahead and all this data came into my office, came into the computer model and I made a subjective evaluation looked at all of these, and I made a subjective evaluation of their data and yes, I agree with their diagnosis of FIP. So that there was that event as to who's going to get into, and it was based not only on the criteria, but on the totality of what was presented. Was it convincing, and we excluded all the effusive FIP cases. Then we had on the ones that we done, we had monthly physical exams, we evaluated them, to look at you know, weight gain or a loss, whether they're still febrile, how they were doing, what their appetite had done the previous month, and they were laboratory tests on a monthly basis.

Okay, let's look at what was included in this study. We had 102 cats overall, and we went ahead and 57% qualified, those that were excluded and a lot of these were quite advanced. I made a special point of not excluding anybody. I was very careful of skewing the data, and consequently cats or 10% of the number that died before they received the polyprenyl, so that it said that the outcome, I did not exclude those that were hit. Then we excluded another 13% as far as evaluating ones that died before they've been on treatment for a week. When an immunomodulator to go ahead and turn them around, you know, we're going to give the equivalent at least a week to work, to have the chance. Those that were lost to follow up. We have one person withdrew their cat from the study after starting on the study. She says, she wanted to give them a holistic approach and went on some herbal medicine and came back in 2 weeks, and wanted to get back on the polyprenyl, she was excluded from the study and we had some that were disqualified for a variety of things, third, we have you know, people that didn't return the forms so that those are the ones that were disqualified which gives us a total of 58 which to work with. What were these cats, we found that we had pedigreed cats. We have about 30% which I know is a lot that it would have been higher than that, but we had 30% of pedigreed cats, because of course the breeders who keep up with the literature were much more aware of the initial publication at the public at large. So this wasn't hollow, those going on pedigreed, as if somebody it doesn't fill out that block in the forms, so we have some that somebody who knew who they were. Now, looking at the forms and FIP that were represented through the

study, a lot of them were abdominal, most of them GI with granulomas, small intestine, granulomatous changes in the colon, a lot of them were seen with varying GI signs, big heavy mesenteric lymph nodes, that accounted for the largest group, we had a number of the people in ophthalmology who are aware of what was going on, we have a number of them where ocular lesions were a principal thing, neurologic signs and others were those that like we say in Tennessee they ain't doing right, and no particular localizing that lesions that we could identify or aspirate and this is where a lot of those probable FIPs came in.

Now, looking at age, fairly typical distribution we went ahead and looked at the 58 qualifying cats, there were 36% of them that were under 12 months of age, and between 1 and 2 years of age. We got another 19% over here, so that comes up to 55% if my math is correct. And then there is some at the older variety of age cats and when we look at the age distribution that we had in our population of cats, with the dry form of FIP, and we look at what was found when we, we have done a survey a few years back looking at the national statistical measure of records from veterinary schools around the country, where they were looking at FIP as a general disease and looking at the percentage of cats in the clinic that had FIP compared to other cats of that age group. We see that 2 to 6 months of age, the FIP ones are counted in for about in that age group counted for about 12, 13% or less than 10. In the 6 to 12 months of age, there's a whole lot of FIP cats than those with other diseases in the clinic.

Again as you can see, equals here somewhere along the 2 or 4 and then as they get older the cats with other diseases, the cats with FIP had lower numbers. Here's the data that we have as far as survivors, and you can see that we get into looking at median survival time, median survival time by definition is the number or purported in which 50% of the cats are dead. So our median survival time was about 50 days and that's a, you know, and you allow that control group we were talking about, it's hard to say what that means. We see that we have a situation where we have cats at various time points that are still alive, so that depending on how long these individuals lived would go ahead and shift that curve to that longest survival time, since this thing was put together last week we have lost two additional cats. One from disease, and number 29 here was killed by a coyote.

So that we got a situation, and you can see, I think this accounts for one cat that's still alive here, past the 12-month mark. So our next step is to go ahead and let the data mature, try to, we'd like to send an email to everybody to keep their cats away from coyotes and more, and see as this curve matures, what our survival time will be. So in the study, the longest survivor we have is at 289 days, so that the when the study was started around the 10th of March of last year. And all the cats of course were not accrued all at the same time. They were accrued over a period of the whole year. The other thing that we see here is that this designates what type of lesions they had, you can see, 29 had neurologic, and this is 29, here's the one that was killed by the coyote, when he was sick he was hanging around the house, once he started feeling better, it was hunting again, it was bringing little presents to the owners on a regular basis. They insisted so that one had been doing well. We had GI, another neurologic, and ophthalmic, a couple more GIs, neurologic, ophthalmic, GI. So you can see that there was a wide distribution of types of lesions of FIP, organ systems involved, that we had in here, so it wasn't restricted to responses in only one.

Now, if we look at the first Kaplan-Meier curve we looked at those that were probable cats, this Kaplan-Meier curve only looks at those that are only the definitive FIPs. So we see the curve one curve quite mimics the other curve so it doesn't seem like the probable ones skewed the curve in one way or the other. We went to look at data on survival times that we have, we know that the survival time on cats, with FIP is quite poor. This study done by Ritz in Germany and most of these cats were wet form FIP, they're not going to do it as well. And you know that they die quite quickly with immediate survival time, half of these cats were dead in 9 days, with the longest survivor they had at 200 days, so they did have, and these were cats treated with the omega interferon, and the one that lived 200 days is now the one that was on the treatment group. Now there is a study done in Taiwan by Dr. Tsai, and they looked at 46 cats, they went ahead and they looked at mean survival time. Mean is the average, so if you have some that live a real long time, you will have most of them that died quickly, but then you had some that lived a long time, and it skews your numbers, so that median is a much better method of doing it. So the time when 50% are still alive, and they found looking at average survival time, mean survival time, that for wet form we got 23 days, and for dry form they got 38 days in the dry form compared to, if you looked at our mean survival time at 81 days. Now, they had a group they called Mixed where they started off as a dry form, and then they developed some effusion and some of them reabsorbed the effusion, and they found that they didn't have any median data. They have a mean survival time of 110 days, which looks very good, but they had two cats who survived 181 days, and over 400 days.

Somebody's got melfinavir here which is an antiviral drug, that's one that I thought might be very interesting to use, in combination with polyprenyl. But they had 5 of the 7 cats; all of these were dead in 19 days. But they did had some long-term survivors, and whether the melfinavir and human interferon helped or not, but it was not with the data we weren't able to compare those cats that we have that were still alive, but not okay. Now if we look at survival by the type of disease that we have, we found that all of those we can see there's a few of them, that are going out here at a year, and here on the gastrointestinal form, but there's really not a whole lot of difference between these various curves. Now the improvement, this seems to be a characteristic here of cats on the polyprenyl; this is an evaluation done by combination of clients and veterinarians where the cats seem to do better. They felt better, they were eating better, and they will be more playful. Very, very subjective type things, and of course the ones that lived over a 150 days were some of the ones that were felt to do the best as per the owners.

So let's look at a couple of these cats that we have, where one of the definitive, this one had, gut lesions, the titers were up in the 1:6400, diarrhea, abdominal masses. This one, the longest one at 374 days. This little kitten must have been a baby picture since it was seen at 11 months old. Again, definitive with uveitis and some of the corneal clouding and some of those things. All these signs resolve and he did quite well for a while, he survived 272 days and it seems like they'll get better, they're going along just about the time that you get ready to relax and say, oh, we got this one that's the corner, some of these were just crash and burn, and it does seem though that when they come to the end, they deteriorate very rapidly. So that while some of these, I think we do improve quality of life in these animals. Another one was definitive with fever, weight loss, kind of activity, just improve well being and this one with a 207 day survivor, and this beautiful kitty here, another of the definitive that was out at 289 days and this is Josetta who is a beautiful bengal and this is the one that was killed by the coyote. And the last we had

on it was 198 days. These are the ones that we have on our hall of fame series, from the earliest ones that we have that we did before we start the Winn study where Grindle, the poster child of five years, and the memory of some of these with others, that were since August of 2009 and this one still alive, this one here, since February of 2010. This one here was alive when quickly studied, another alive at 18 months, so we aren't seeing large numbers of cats being long-term survivors. We are seeing some. So conclusions at this point we believe that the polyprenyl immunostimulant improves the well being and probably survival that cats that had been stable a long time, or that die or euthanized seem to do well for a while and then they fade out quickly in terminal stages. We want to go ahead in future studies and look at the polyprenyl immunostimulant with either antivirals, looking at some of the small interferon RAs that go ahead, that might stifle the growth, I think you have to control the virus, so that the combination therapy make sense, we need to explore the other uses of the polyprenyl, I want to go ahead and do more of course with rhinotracheitis and see what it will do with cats with ocular Herpes and we want to go ahead and do some more studies on the immunology. And I want to thank specially Gina Galvin who's the technician whose part of her salary was funded by the Winn Foundation to try to keep track of all of these. Tanya Curtis, Arthur Sas, all the veterinarians all that's in the cast and participated. Thank you.