



WINN FELINE FOUNDATION

For the Health and Well-being of All Cats

637 Wyckoff Ave., Suite 336, Wyckoff, NJ 07481 • www.winnfelinefoundation.org
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2013 Winn Symposium Transcript Application of Stem Cell Therapy and Use of Mirtazapine

June 27, 2013 (published)

Jessica Quimby, DVM, PhD, DACVIM

Steve Dale: Good afternoon, my name is Steve Dale, I am on the board of directors of the Winn Feline Foundation which is something I am so proud to say I am because I think I have something in common with everyone in the room that would be I kind of like cats. Does anyone have a cat?

You know I am tired of cats getting kind of the short shift. Here is what I am talking about, more cats are given up to shelters than dogs are. Fewer cats are identified, we tend not to microchip cats or put IDs on them, you know collars with a tag saying who they are. Fewer people actually go to a shelter to look for that lost cat than a dog, which is shocking. When they do they are not as likely to find the cat that is their cat. More cats are just let outside. We tend not to do that with dogs. I mean let outside, close the door and we do not want them back. Not any of us in this room, I am talking about just people in general. Cats do not see the veterinarian as often as dogs, about half as often as dogs. Yet we have more cats in America than dogs so at some level cats are man's best friend and I am just kind of tired of cats being the Rodney Dangerfield of pets. We need to change all that and give cats the respect they deserve.

That is part of the reason that for 383 years (laughter) the Winn Feline Foundation has been holding a symposium. Of course I said that in cat years but we have been doing this for a very long time. I will talk a little about the history of all of that in just a little bit. How many of you have been to one of our symposiums before? Oh my gosh, everybody in the room. For those who did not raise their hands, welcome! This is a good thing, it is a great learning opportunity. Some number of years ago we thought what a great opportunity to get CE credits for veterinary professionals; there are veterinarians in the room, veterinary technicians, a special welcome to all of you. Homeagainheroes.com (I think it is .com) are having a contest where you can nominate a hero, anybody who in any way is a hero for cats or dogs, it doesn't much matter, for companion animals. It can be a first responder. It can be a shelter volunteer. It can be anyone. Well for those veterinarians in the room you are heroes in my world because you save animals all the time.

This is kind of like the 411 on what the Winn Feline Foundation has been doing since 1968. It was developed originally as an idea from the Cat Fanciers Association. So Cat Fanciers Association people, thank you! You made it possible for us to save lives and that is what those of us who are involved in the Winn Feline Foundation, that is all we want to do. We want to make a difference for cats. I like dogs, I have two myself at home. A lot of you have dogs, right? Nothing wrong with dogs but let me tell you a little secret. The Canine Health Foundation, they raise millions and millions of dollars.

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They do, and for every (I am making up this number but is not too far from the truth) million dollars they raise we struggle at the Winn Feline Foundation, the only organization on the planet that solely raises money for felines; we struggle to raise \$300. It is easier for whatever reason to raise money on the dog side. That has to be changed because cats get sick too. We have made a difference for all of those who kind of raised their hand when I said do any of you have a cat? Of course you kind of raise your hand in this group, right. Lots of cats over the years, probably breed cats for most of you in this room. This is a small partial list. I could do a whole hour on just what the Winn Feline Foundation has done in all of the years the Winn Feline Foundation has been there. Sometimes what we do is the seed work.

We do the work and then another organization, well for example with feline leukemia. No one knew really what the feline leukemia virus was or what it did or how it worked. It was called this like, rinky lymph node disease. No one even had a name for it. It was with the Winn Foundation seed money that researchers and investigators were able to figure out in what in fact feline leukemia is and how cats got it and it was Morris Animal Foundation that then came in once that knowledge was learned and said okay now we can create a vaccine. We often work in tandem in a sense with other organizations but there is not a cat that any of you have, and I would sat there is not a cat in America that in some way, probably in lots of ways, definitely in lots of ways has not been changed or impacted by the Winn Feline Foundation. Do any of your cats eat? Even the very food that we feed our cats, unless you make it yourself and even then what we know should go in that food, that has been studied by research that long ago, before my time, that the Winn Feline Foundation funded. I am glad you are here. A part of our mission is that funding, but a part of our mission also is education.

You know what I need, oh this is so embarrassing. Yes, thank you. By the way, the board members or many of the board members of the Winn Feline Foundation are here. That is Vicki Thayer, president of the board of directors. Dr. Thayer, stand up please!

The other board members of the Winn Foundation, my colleagues on the board, please stand up! Come on!

It is truly a wonderful board; we all work for the same thing. Every year that I have been at this podium, I have been lucky enough to be able to acknowledge my colleagues on the board. What I have never been able to do until now, this very second is acknowledge maybe the most important person in the room! For the people that are not in this room they can later watch this because we have a videographer, Dr. Thayer's husband Bob is right over there!

You know there are not many cats and we are going to learn in a second whether it is true or not what I say as Dr. Quimby will verify this or not. Maybe there are not any cats that are lucky enough to live beyond the age 8, 9 or 10 that do not have some sort of renal insufficiency. We thought this is an important topic to talk about because it affects so many different cats. The Winn Foundation is working very hard to help researchers learn more about this. Following her graduation from the University of Wisconsin, Madison School of Veterinary Medicine, Dr. Quimby completed a small animal rotating

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internship in Sacramento. She then pursued her interest in feline medicine in private practice at a feline exclusive clinic in Grand Rapids, Michigan. Wanting to learn more about medicine and the advance in the state of medicine for cats in particular, Dr. Quimby completed a small animal internal medicine residency at Colorado State University where she continued to concentrate on feline medicine. During her residency she specialized in feline kidney disease (no surprise) and also respiratory disease. She recently completed her PhD exploring several aspects of feline kidney disease including palliative stem cell therapy and renal aging and is now a faculty member at Colorado State University. Please help me welcome Dr. Jessica Quimby.

Dr. Jessica Quimby: Thank you very much. It is an honor to be invited to speak here at the Winn Symposium. I am just going to take a moment to pull up my presentation.

What I would like to do this evening is talk a little but about some of the research that we have been doing at Colorado State University. As mentioned with that fabulous introduction what I have been focusing on in my life is basically the study of chronic kidney disease in cats. This is something that I have always been very passionate about and there is so much work to be done in this area, I will probably be doing it for the rest of my life. This is what we have done so far with the help of the Winn Feline Foundation and I wanted to talk a little bit about two of our big programs of study that we have going on at this point in time. With that like you mentioned, it is a very, very common disease in elderly cats. When we think about the number of cats that we have in the US, it is estimated to be approximately 24 million cats that probably suffer from this disease. We have literature that tells us that about 30% of elderly cats are affected by chronic kidney disease and honestly I do not believe those numbers. When we see what the cats that come to us at Colorado State and when we talk to practitioners, it is actually hard to find an elderly cat that is not affected to some degree by this disease so as we are taking better care of cats with better medicine they live longer and they tend to get chronic kidney disease if they are not affected by something else first. This has really become a major management issue for us. Unfortunately we do not know why it is that they are getting chronic kidney disease. Why is it so common in elderly cats and why is it that we cannot do anything for them? We need to find out what is causing this disease and also what we can do.

As many of you know, there is not anything that can be done about the disease other than a kidney transplant. It really becomes incumbent upon us to find additional treatment modalities that we could use. What can we understand about this disease so that we have a better way to treat them? That is really some of what my research has been doing is to look for additional options for these animals.

There are two projects I would like to talk about as you know. We will talk about the stem cell program and then we will talk about supportive care with appetite stimulants as they get this disease. The stem cell program is I guess our hottest topic. It is something that people always ask me about, what do we know about this disease and why do we think that stem cells potentially can treat this disease?

So just a little bit about why, where did this idea come from? We were sitting around one day, you know, contemplating kidney disease as happens a lot at CSU and thinking to ourselves, what can we do? What is in

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the literature that would be novel that we could apply to this disease process? There is actually a huge body of literature about stem cells and acute kidney injury and chronic kidney disease. There are literally hundreds, at this point in time, of different studies in rodents that tell us that stem cells could be beneficial for the treatment of chronic kidney disease. When you read some of these articles they are really compelling. Animals with acute kidney injury get stem cells and they live and the other animals do not, it is that simple. Animals with chronic kidney disease have an improvement in their numbers. Their creatinine improves. They have decreased proteinuria, if that is present, and they have decreased inflammation within the kidney and also decreased scarring or fibrosis within the kidney. This is all in rodent models. The question is, can we apply that to our cats and help them out with their disease process. Then the question is well how would stem cells be helping out the chronic kidney disease or the acute kidney injury process? A lot of people always ask me, is it – are we making new kidneys? Well unfortunately we are not making new kidneys. It is actually an effect most researchers now believe is what we call paracrine effect. So there is cellular messaging that is happening within the kidney. There is all this inflammation and scarring that is happening in the kidney as the disease progresses and there are little messengers that are telling the kidneys ‘Okay you just need to stop being inflamed, you need to repair’ and the stem cells are very helpful for that process. That is really what they are doing and we call that the paracrine effect of the stem cells. They are anti-inflammatory and they also protect vasculature. So when your kidney is diseased the vasculature, those little blood vessels become divorced from the cells that they are supposed to feed and that is part of the process. The stem cells are thought to help with that and that is part of what we really think their goal is or the effect that they would be having in the kidney.

We read all of this literature and we say wow this is fascinating, how can we apply this to our cats?

Why, and I think a picture is worth a thousand words and I want to show you these images so you would have an idea of what we would hope the stem cells would be doing. This is what we see when we look at interstitial inflammation in the cat kidney. On the left we have a normal cat and there are all the beautiful little tubules and you do not see a lot of purple in this picture. On the right this is chronic kidney disease and all of the purple cells that you see are inflammatory infiltrate coming in to the kidney and destroying the architecture in there. That inflammation is then later replaced by scarring once the normal architecture has been destroyed. We see this as they progress through their disease. We have been looking at the histopathology. We have been looking at these kidneys throughout the stages of chronic kidney disease. In this picture blue is fibrosis or scarring. This is what happens as cats go through the stages of their kidney disease. They start out and there is only a little bit of blue. Then as they progress more and more inflammation and more and more scarring occur until the kidney is pretty much replaced with tissue that does not function. Our goal is to really slow down that process and that is what we are after with this particular treatment.

With that in mind after having read these articles it was honestly really my idea to start stem cell research at Colorado State to study chronic kidney disease. That was the whole point of our program as we read these articles and we were really, really interested in this, but no one had done this. So we had to start somewhere learning about

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feline stem cells, learning where do we get feline stem cells from, how do we grow them. We were the first feline stem cell program in the country and honestly we could not have done this without the Winn Foundation because when you ask for money for this type of thing people say ‘You want to do what with stem cells?’ So it becomes a little bit of a problem to start a program from the ground up with something that is really innovative and we were lucky enough to get support for that project and this has started off our entire program for stem cells at CSU.

We wanted to learn more about feline stem cells in general and we also want to basically, we have worked through our series of pilot studies looking at what could these stem cells do for cats with chronic kidney disease.

I am going to talk about three different series of pilot studies that we have gone through looking at putting the stem cells directly into the kidney. We want to find a way to easily give them to cats so we have worked on cryopreserving them or freezing them so we can just thaw them and give them to the cats. Also, cryopreserving the fat so we can then thaw the fat and make stem cells and give them to the cat. I will explain a little bit more about that shortly.

I do also want to mention that one of our other visions of the program was to look at stem cells for the treatment of asthma and IBD and I am really proud to say that we now have collaborations with two other researchers who are also Winn funded that are looking at stem cells in asthma and IBD.

Where do we get the stem cells? This is always the question. These are adult stem cells so this does not involve embryos. I once had a woman ask me, are you grinding up kittens, Dr. Quimby? I said no, we are not grinding up any kittens, I swear! A lot of this work originally started in horses in veterinary medicine and horses are very large and it is very easy to get bone marrow from them. They get it from the sternum. It is not fun, for the veterinarians in the room, to bone marrow a cat. So this is where all the body of knowledge was and we started off doing bone marrow derived stem cells. Not fun but then adipose has gained in popularity. Obviously this is something that we have a lot of! Not an issue to get adipose tissue. We also have several other companies that are now looking at shall we say spare bits that you can get stem cells from. I believe one of the other universities is going to be looking at, once you spay and neuter a cat can you use the spare bits for stem cells for that animal later. That would be another source. For us we have decided upon adipose. One of the reasons for that is in our original studies we actually compared; when I was getting that bone marrow, in the elderly cats it is very hard to grow the bone marrow. It is very hard to obtain the bone marrow and I thought this is just not practical. We need something easier. We have some really healthy spoiled and a little bit plump research cats and they would provide the fat for a lot of our studies and I will talk about that. The other thing we discovered was the adipose grows way more stem cells than the bone marrow does. So, you can get a little bit of bone marrow or a little bit of adipose and it grows many, many more stem cells much quicker. That is what this graft actually shows. In the red you get a much higher yield from the adipose and it is easier. So for cats adipose is the way to go. And who needs the little paunch, so we focus on the paunch and we give our research cats a little tummy tuck before they get adopted and they give us stem cells. That is where they are coming from.

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The next big question we get is, 'Are they stem cells?' Well how do we know it is a stem cell? This is a question that is something that understanding the product that you are using is really important especially when we have a lot of companies who are providing stem cells for use in clinics. I want to explain a little bit about how we know it is a stem cell.

First of all it seems almost like science fiction to me honestly to this day. You take some fat, you grind it up and you put it in a flask and several days later stem cells stick to the plastic and you start growing stem cells in culture. It is an amazing thing to see and you get this beautiful little arrangement of cells that stick to the plastic. For whatever reason they like to stick to plastic and we use this to our advantage to isolate them in culture. There are also different ways that we can characterize them with little surface markers using flow cytometry on the surface of the cell. Those markers are not specific to stem cells so we do not find them to be very useful. We do it for the sake of the science of saying okay we characterized our stem cells and we know they are positive and negative for what they should be but it is not nearly as entertaining as the other thing that they can do which is to differentiate into different cell types. If you want to say 'I have stem cells' you need to be able to show that your cells can actually be turned in cells of other different lines. Again this is a thing that really is fascinating to me.

In the lab we take our typical stem cells and we can then make them by adding certain substances to their media turn into cells that produce fat, calcium or bony constituents or little tiny cartilage globules so literally you have got these stem cells and they will start making little blobs of cartilage right in the flask. Then you can collect this material and test it to say look we have cartilage that is being produced by these stem cells.

For them to be able to do this, this is called trilineage differentiation, that tells you that you have a stem cell that could be all these different cell lines and then you can prove the cells. With the cells I am working with that is what we are talking about, they can do all these different things. They jump through their little hoops and now we know who they are.

I do want to make sure that stromal vascular fraction is basically, that people understand what that is. When you process the fat and you spin it down you have what we call stromal vascular fraction. This does have stem cells in it but this is often the product that has been use commercially as a stem cell product. So if you harvest fat and you send it away to a company and it comes back to you 48 hours later, you have stromal vascular fraction. You do not have isolated stem cells. That takes 7 to 10 days in culture, right. It takes a couple of weeks to grow them up. There are stem cells in that population but there are also other cells, other than stem cells and interestingly enough we just do not know enough about this particular substance. There have been studies that have shown that it too can be helpful for disease. We are not saying that this product is not a stem cell product, it is just different. It is important for people to understand that there are different types of stem cell products out there and I hear everything being called stem cells and that can be a little frustrating for us because there is a difference there.

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What were the goals of our studies. We wanted to create a feline friendly treatment and what I mean by that is it has to be not some big convoluted thing that is hard on the cat that involves multiple procedures. I really want something that is going to be clinically applicable. That is what we do is come up with something that will actually work in real-life practice. I was in practice, I know what it is like and it has to be reasonable if it is going to be a treatment that is actually going to work in terms of logistics and it also has to be effective. Those are the two biggies, it has to be logistically possible and also efficacious and that is really what we are going for. Our hypothesis is of course we do not want to hurt or kitties, we want to make them better and we also want something that is easy to give and it helps out with their chronic kidney disease which is really kind of a tall order.

I am going to describe the series of pilot studies that we have gone through looking at different ways that we can give stem cells and the potential effects of it on the cats and their kidney disease. When this first started to come out, everybody thought, there was a big argument in the literature about can you just give the stem cells willy-nilly to the cats and will they find the kidneys or do you have to put the stem cells in the kidneys. Because we were unsure in the literature and the experts in stem cells were a little but unsure at that time we chose to do our first pilot study by putting the stem cells directly in the kidney. Also because of the current literature at the time we elected to get the stem cells from the cat with chronic kidney disease. This is autologous stem cell therapy. We would take the bone marrow or the fat biopsy from the cat with chronic kidney disease. We would grow those samples up in the lab which of course takes two weeks and then give it back to that patient. We did both bone marrow and adipose derived and when you do a procedure on your elderly little patient there is a lot of praying involved in making sure that those cells grow. So that was stressful. I thought well maybe this is not the best way to do this. We will look at other things in the future. Then we would give those cells back into the kidney with ultrasound guided injection. We dose escalated in this study. All of the cats actually did really well and you would think that this is a little bit more of an invasive procedure but if it were to work this would be great. What we really saw in this study was the cats had mild improvement in their numbers and we looked at their GFR or there glomerular filtration rate (excuse me) and some cats really had a significant improvement on an individual level in their GFR but it just was not enough for me to say it is worthwhile to go through this whole procedure of collecting their bone marrow, their adipose and injecting into their kidney. The disadvantages really kind of seemed to outweigh the benefits and when we quizzed the owners who were involved in this first study, they said 'Yea, you know it could be easier.' What could we do to improve the feline friendly part of this whole thing?

Basically there were too many procedures. We were doing GFRs for the sake of science, you would not do that in practice but we needed to determine whether it worked or not. Then it is hard for these elderly kitties to go through that procedure of collecting the fat. They have to be sedated in order for the ultrasound to happen to put in into the kidney. So what if we could just give it IV? Actually another paper came out at that time that said giving it IV to the rodents was very, very helpful. We decided to change our program and go in that direction. In addition the elderly kitties posed a problem, it is not fun to bone marrow an elderly kitty and also the cells just do not grow as well as I showed you.

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This is Frankie and Frankie is actually the kitty who was in our first pilot study that started Frankie's fund for feline stem cell research, her mom did actually, not the cat of course. Frankie was a little bit plump when she was first diagnosed with kidney disease so we decided to try the adipose route at that point in time and her cells grew fabulously. We compared the two in those cats and that is when we decided to transition to the adipose. It was much less nerve wracking if you will to make sure that you were able to get a treatment for the patient. The other thing though would be, I thought to myself, why are we trying to get these stem cells from the elderly patient. Wouldn't it be better if we had fat donors? This is where our plump little research kitties come in. It would be much easier if we could use allogeneic therapy and have cells just waiting there for them to use and then the elderly kitty then would not have to go through that procedure and then you could treat acute kidney injury or lily toxicity or some other thing where we did not have time to wait for the cells to grow. So, that has been another big focus of our program is trying to arrange that. So we actually switched to allogeneic stem cells at that point in time and that is where all of our research kitties like I said get a little tummy tuck and they do very well with that. It is pretty noninvasive for them.

This is the initial pilot study that we had five kitties that were involved in this particular study. Again we now were going IV and the protocol, this is our new study. We switched to a repeated IV administration of stem cells. Now that it is easy we can easily give it in a repeated manner and see how they do with repeated injections. So they would get stem cells once every two weeks and then the other big question everybody asks us is well how long does it last if we give stem cells, is that going to last six months? Will it change things and help for a year? Then we actually gave them once monthly injections after that to see if any effect we saw would last from that. Realistically again from a logistical standpoint giving them an injection IV every two weeks is not logistically realistic. We also did clinical monitoring in these kitties and the other big thing I want to say is how do you know what is going on in the kidney? So because we cannot do a kidney biopsy, it is not safe, we have actually developed a program looking at cytokines or mediators of inflammation in the urine and we used this as a proxy to determine if we are affecting things in the kidney. Can we make that inflammation in the kidney better? So that is the other thing we are measuring. All of this is pretty much true for the pilot studies that I will talk about with little amendments.

This is Colby, he was our poster child for the IV study and he was owned by one of the gals in the VTH. We actually saw a significant decrease in creatinine in this first study and we were very excited about this. It was a relatively small decrease but all of the cats had it and that was kind of a really exciting point for us. We elected to go on at this point in time. All of the cats did very, very well. None of them had any problems so this is where the people start to say well you are giving someone else's stem cells to these cats. How are they not having a reaction? Stem cells are actually immunoprivileged and you can give some cat stem cells to cat B and not have a problem because the immune system does not see those stem cells. None of the cats had any problems and actually in some of the papers they give different species of stem cells to rodents. So they will take human stem cells and they will give rodents human stem cells and there is no reaction. That is how immunoprivileged they are. We did see that small but significant decrease in creatinine and also in urinary cytokines and so this as a first IV pilot study was exciting but then with the creatinine you really

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cannot tell because if the cats change weight their creatinine might also change so we needed a little bit more definitive measure of kidney function. Then unfortunately the effect seemed to wane over time so once we went to the monthly injections their creatinine kind of popped back up again so we thought 'Oh well, maybe it does not last as long as we would like it to.' All of the kitties in all of the studies, I should mention we are following until the time of euthanasia so in the end we will be able to look back and see who was in studies and did they live longer than average that we would expect for chronic kidney disease patients.

Then we decided to move on to pilot two and we added in an iohexol clearance test which is another measure of GFR that can be done easily within clinic and we increased the stem cell dose because there was some indication that a higher dose might be better. This is Truman, she was one of our research kitties that got a tummy tuck and got adopted.

Pilot two was five kitties and in this particular study we actually did not see a significant change in the creatinine or in the urinary cytokines so it kind of give you an idea they were straight across the board. Their urinary cytokines did not change over the course of the study either but we ran into another issue. I am sorry, this first. The one thing that always gives us hope is no matter what pilot study I am in there are always some cats that do fabulously. Even when their creatinine does not change, the owners invariably report, they are like a kitten again! They are leaping around the house and doing really well. When we look at the GFRs, the three dotted lines are cats that did not get stem cells, they were the controls. We have cats, like cat 1 who had a 75% increase in their GFR. That is huge if you actually have hardly any kidney function to start with. As a general rule the control cats never vary more than 10% so every once in a while in these studies we have a cat with a GFR that really improves as a result of the stem cell therapy even if their creatinine does not seem to change. I honestly do not have a great explanation for that other than it is what continues to drive us forward.

Variable responses in their GFR, however these were the cryopreserved cells and when we increased the dose we started having problems with giving the cells. Some of the kitties would get increased respiratory rate and others would have nausea and vomiting as I was giving the cells. It actually turned out that this was a problem with cryopreservation, not the stem cells themselves because we then subsequently gave fresh; we have never had any problems with fresh cultured cells. But, kitties like to vomit. They will vomit when you give them substances and so something about cryopreserving the cells and then giving it to the cats be it the cryopreservation, I mean we washed them afterward, we were very, very careful to wash out the freezing media and everything. The cats still would react to these cryopreserved cells so some more grey hairs were created during this pilot study and we decided that we needed to change things because again, rule number one, do no harm. We need to be effective but we needed to not harm kitties. Then in a moment of one of those serendipitous scientific moments, I got really sick of processing fat one day and I just threw it in the freezer. We had a lot of fat to process and we discovered that you can actually just freeze the fat and then thaw it out and grow the stem cells up fresh which is what we do now for all of our protocols so you have fresh cultured cells that you can use and the cats have absolutely no problem with them. It takes about 10

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days to do that in the lab. We also in this pilot study three we added in nuclear scintigraphy. With scintigraphy when you are looking at GFR you can tell the difference between kidneys so you can actually take the GFR of each kidney to see, a lot of these kitties have big kidney, little kidney syndrome right, where one has been injured and the other has not and it has gotten bigger because it is trying to recover, a little hypertrophy. The question was do the stem cells help out the big kidney more? Do they help out the little kidney more? Or does it not make a difference so that would give us a little bit more information. We kept the dose the same because the question that we really wanted to answer with the pilot study was what is going on with those cryopreserved cells. If we give these fresh cultured cells and then the cats not have any problem that would answer that question for us.

Pilot study three again was five kitties and I should say that these pilot studies are so small because one of the caveats or one of the requirements of being in this study is that they have to be an elderly cat with only kidney disease. That is kind of a problem when it comes to enrolment. We screen a lot of cats and the reason we have to be so stringent about it is because the stem cells are attracted to everything so if you have IBD or if you have skin disease or you have some other condition your stem cells are going to be attracted to areas of inflammation or infection so nothing else can be going on if we are going to get a good idea of whether or not they can really help out with the kidneys. We screen a lot of kitties and that is why our pilot studies tend to be on the small side but it gives us a good idea of what is going on and each time we change a little something.

With pilot three again we did not have a clinically significant change in serum creatinine or the urinary cytokines but the same thing happened with the GFR. We had kitties that had a 63% increase in their GFR and a 91% increase in their GFR. The GFR in the one cat almost doubled what it had been before. Then we have one kitty that went down. Well this tells you how sensitive GFRs are to things because unfortunately Harley was constipated that day. He was having issues. Part of the stem cell study is that you cannot have any treatment changes and they have to be stable chronic kidney disease cats but that is almost like an impossible thing to be a stable chronic kidney disease cat. Throughout the course of the eight week study, this was a cat that had intermittent bouts of constipation unfortunately on the day that he came in he was constipated and there was also a snow storm so mom brought him up the CSU the night before and stayed in a hotel. Unbeknownst to me, I probably would have said, let's just reschedule. So poor Harley was constipated, stayed in a hotel overnight and probably did not drink what he normally drinks. That has the power to change what his GFR is unfortunately. So clinically he had no change but those GFRs are really sensitive and that is unfortunately one of the limitations of this type of measurement. Again 91% increase in GFR so this is really interesting and this is why we keep moving forward with this.

Conclusions from that, effects are variable. Quite variable in terms of what they can do. We did not see administration side effects when we used those fresh cultured MSC and that is now the way that all of our pilot studies move forward. We freeze the fat and we grow the cells from the fat and everybody does fabulous at home. As a note, we also think that stem cells are good for arthritis so some days I think we are just treating arthritis and everybody is running around like kittens feeling fabulous because their arthritis has been treated so it could also

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be very good for that. One of the cats that did very well had bad lumbosacral arthritis and I really do believe that probably made a big difference for him. We have been wanting to do an arthritis study but it is very hard to assess in cats and although we have force plate technology at CSU the cats just slink no matter what when they are in the clinic so you really cannot assess how they are doing with their arthritis, as an aside.

Future directions for the stem cell program: We still have like an entire; I swear there is like a 10-year plan in terms of chronic kidney disease for the program at this point in time. We are currently in the middle of a placebo controlled clinical trial with those fresh cultured stem cells and the one thing that was noted was that as the amount of stem cells may matter, we are trying to enroll cats with a lower level of chronic kidney disease or earlier stages. Because if you remember in those pictures I showed you, once they get to the end stage, it is already scarred. It is already fibrosed and we cannot turn that process around. We need to prevent it from happening so we are enrolling more stage 2 kitties. Well I have some 7 and 8 kg, almost 15 to 20 pound stage 2 chronic kidney disease cats somehow and so when they are getting a flat dose of stem cells that does not have the same effect as an under 2 kg chronic kidney disease cat, so this new pilot study actually administers the stem cells by weight so that everyone is getting per mass the amount of stem cell they potentially would need.

Further areas of research we will be looking at is actually going back to giving the stem cells near the area of the kidney so in the retroperitoneal space. Again, that would be something to get them in the area of the kidney. Would that be less of a problem? It is much easier to do that injection than it would be to do the intrarenal injection that we did before.

Then we continue to strive to know more about feline stem cells. What is the best way to cryopreserve them? What else can we apply them to? How do they work and how can we help out cats and all of their inflammatory diseases because they like to react to everything. How can we help them out with stem cells and what is the potential for that? So that is really the focus for the future direction of the program at this point in time.

With that I would like to transition over to talking about mirtazapine which is really one of my favorite subjects as well as an appetite stimulant and anti-nausea therapy for cats with chronic kidney disease. As I said this is a topic near and dear to my heart as I am sure probably everybody in this room has had a cat with chronic kidney disease and we spend all of our young years as cat owners getting them to not be fat and then once they get one of these terminal illnesses we spend every single day trying to get them to eat and it can be really challenging so this is where we can really get some help and help some of these patients out. What is the importance of nutrition and why do we worry about it?

Obviously they need to eat to live, very basically. It is really, really important for immune function, healing and strength, keeping up their attitude and their liveliness at home and there are actually a number of studies that show that a poor body condition is linked to a poor prognosis and this is true in any species and especially with chronic kidney disease. So once you become very, very frail, even as a human, your chances of doing well with dialysis are much less and if you are a dog or a cat with chronic kidney disease you will lose body condition, you lose energy and if you have another uremic crisis and you end up in the hospital, you just do not do as well.

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You have lost that energy reserve to get you through crises and it ends up being the end of the road for those animals. Really the biggest thing to me is quality of life. This is really what cat owners see and struggle with every day and I have people who literally sit in the exam room and cry about the fact that they just cannot get their cat to eat and that really is just so moving to me and if we can do something to help with that process it is really quite powerful. It is very, very stressful and there have been a couple of quality of life surveys for cats, not for kidney disease but for other diseases. That is one of the number one things that people say is that just getting the animal to eat is just such a stressful thing for them. In the end this is why they get euthanized. If they keep eating and they keep their body condition up they keep going and they do much better with their disease. That is really the goal, the underlying tenant of this work is to help them out with that.

Just a little bit about mirtazapine and why we thought this would be a good idea to look into this further. It is actually an anti-depressant in people and strangely enough the major side effect is sedation which is not the case in cats. Often if owners have experience with this drug, they say “Ooo” that does not seem like a good idea and I say well cats are not people and it is different. We have different side effects. The major thing that we are interested in is this 5-HT₃ receptor antagonist action. This is the same receptor as ondansetron and dolasetron or Anzemet so an anti-emetic and that is a really important point for this drug.

In humans and other species it has been shown to have the side effects of appetite stimulation and antinausea. If you are depressed and you are taking this drug and it makes you eat a lot, not really the best thing in the world right! So it did not turn out to be the best drug for depression because it just makes people eat and they get more depressed and it is a bad cycle. But, if you have cancer and you are depressed and you are nauseous it is a great drug and there have been a lot of studies that have shown that. The anti-nausea effects are actually quite fascinating so people who go under anesthesia and get really, really sick from anesthesia, some of you may have personal experience with that. There was a great study that showed taking mirtazapine before the anesthetic event greatly reduces the problems that people have with post anesthetic nausea. I thought that was a really interesting study.

Previous work that was out there that made us go hmm. There was a little open trial that was published and this has now gotten into VIN and everywhere else and this is the mantra that we are trying to fight against, 1/4 tablet every 72 hours. The thought behind that and this was extrapolated from human medicine. There was no data behind this recommendation whatsoever. Dr. Lunn and I gave this drug and we said this works fabulously. This is the best appetite stimulant I have ever seen but there is no way that 1/4 tablet every 72 hours is the correct dosing. We need more information. The reason why people thought this might be appropriate is because the way the drug is metabolized. It is glucuronidated in the liver and cats are very bad at that so it was thought, we need to give it less often because of this. Again there was really no information about pharmacokinetics in cats to say that this was the right dose so this was kind of the gauntlet or the glove thrown down for me. We need more information. This is a great drug but I really feel like we are using wrong. In addition to that my end goal was to use it for chronic kidney disease cats even though we use it for a lot of our patients at CSU. We know that renal disease delays its clearance by about 30% in humans and the question was, well if that is the case what is the effect in cats with chronic kidney disease and do we have to change the dose to make up for that?

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The overall aim of the mirtazapine project was to investigate the use of mirtazapine as an appetite stimulant and we were aiming for chronic kidney cats. Because we knew nothing about the drug we had to start with normal cats and find out about how it worked in normal cats before we could get to the chronic kidney disease cats. Our hypothesis was that it would help these cats with the management of their disease. So we have gone through and over a series of about five years we went through a series of studies again. We did pharmacokinetic studies in young normal cats and this was a very clinical study so I basically took the doses that people were saying to give 1/8 and a 1/4 of a tablet which is 1.88 mg and 3.75 mg if you do the math and see what are we finding when we actually give those two tablets. This is a real life study and then we looked at daily dosing in young normal cats when we discovered that the pharmacokinetics was actually a lot shorter than anybody expected. Then we also decided that the high dose was a bit too much and I will talk about that in detail in a bit. We then tested it in normal geriatric cats and chronic kidney disease cats at that lower dose, that 1/8 of a tablet dose. We also because some people would say it is not an appetite stimulant, it does not work, you know I do not believe this. Then we actually had to show that it was an appetite stimulant. In young normal cats, we compared the placebo to the 1/8 of a tablet to the 1/4 of a tablet and basically recorded how much they would eat once they got that. Then last but not least that we have just about to be published is our chronic kidney disease clinical trial which is a placebo controlled blinded crossover study looking at the effect of this 1/8 tablet every other day dose for three weeks in our chronic kidney disease patients.

When we go back to the pharmacokinetics what did we learn from this. We did all three of those groups and this graph actually looks amazingly just like the graph in the human literature for humans. Basically what we saw is healthy cats are on the blue dotted line, the half-life was way shorter than anybody would have imagined. The half-life was 9 hours in cats and it is actually 30 to 45 hours in humans and it is administered daily. So right there that tells you that every 72 hours, there is no drug left by 72 hours. In 9 hours they have gone through half of it. The 72 hours part is pretty, not really what we were seeing but then if you are a geriatric cat in the green actually that does extend the half-life of the drug in your body. This is true in humans as well. As you get older your enzyme systems shift and so things change and often you metabolize drugs slower so in our older kitties we need to pay attention to that. Then having chronic kidney disease slows the processing of the drug even further so this is time to excretion right. As this goes up on the graph, that means it takes longer and longer for you to get this drug out of your body, so we discovered there was this nice movement as you add on disease processes from your healthy young normal cats to your chronic kidney disease cats and this is where our recommendation for the dosing came from. In healthy young cats it could actually be administered on a daily basis according to this information and so for cats that have no kidney or liver dysfunction, maybe they are boarding and they are not eating or they are having some other psychological breakdown; you could mirtazapine to keep them eating or if it is a pancreatitis cat this how we use this drug. Once we get a little bit older or we have chronic kidney disease then the half-life becomes more like 15 hours and the effect really does seem to last for up to every other day and I have some owners for whom the effect is so obvious some owners will give it every 36 hours instead of every 48 and they time it down to the minute.

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In general people have been very happy with that dosing scheme.

What about the pharmacodynamics study, what about the actual dose? Which dose do we use and does it work. The pharmacodynamic study, many clinicians, student and people who work in the VTH brought in their kitties for the eating study. It was kind of like a little spa treatment really. Many of them enjoyed it very much. They had three separate one day stays in the clinic. They received in random order the placebo, the 1/8 tab or the 1/4 tab. Everybody was blinded, nobody knew what was going on and then we recorded hourly after they got this treatment how much food they ate. We would weigh it, how much they vocalized – meows per minute. Social interaction, were they hiding at the back of the cage, were they up at the front of the cage, you know rubbing, head butting and their activity in general. This was a pretty fun study I have to admit. Counting meows per minute was one of my favorite study parameters. Interestingly enough we actually saw that there was no difference in the effect between the low and the high doses. If you gave the 1/8 tablet or the 1/4 tablet they did not eat more if you gave 1/4 tablet but they ate a whole lot more than they did if they just had the placebo. In addition to that we had one cat that ate no matter what. He did not care and then we had one cat that shivered in the corner no matter what so they are cats right. They do not always cooperate but this is a very realistic representation of cats at large so we see no differences in the doses but we do see more side effects at the high dose and this was our clinical impression as well. Oh my goodness!

Sometimes we have owners who say mirtazapine is evil. I gave this drug and my cat is running around the house going meow, meow, meow, meow and yes one of my cats is the same way. I cannot give this medication in the evening and she even needs a lower dose. She gets 1/16 of a tablet and she is still a little bit on the edge. It is an individual cat thing. So if there is no difference in the efficacy we should really be starting with the lower dose particularly in our chronic kidney disease patients so we have less side effects but similar efficacy. That was really what that study gained us. In addition to saying for sure this drug is an appetite stimulant.

Last but not least we have the chronic kidney disease clinical trial. This was like I said a placebo controlled study so the owners did not know which the cats were getting and then it was crossed over so they would do one treatment and then the other. I asked them to just please be objective because once you give the mirtazapine it is clearly the mirtazapine because it works pretty well. There is this little thing at the beginning of the packet that says, 'Okay we realize that once you start giving this drug you are probably going to know placebo versus not but carry on, please finish the study and please be as objective as possible.' They got the 1/8 tablet dose every other day for three weeks and then they crossed over. Every day the owners had to keep daily logs about the eating, the vomiting, is my cat having a happy day, a not happy day. What is their quality of life like and we recorded all of that information. They got a physical exam, weight and body condition score before and after. Much to my chagrin only 11 cats completed the study. Once again we are very stringent with our studies and we screened 172 cats for this study. Then 16 cats enrolled in the study in the end and 11 made it through but this kind of tells you how much work these types of clinical trials involve. The problem is that these cats could not have any other diseases that would affect their appetite. If they are hyperthyroid or if they had IBD or any other concurrent disease potentially it would confound the study

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results. So, because we are trying to have a proof of concept with this drug it is important that it be a really clean study. We realize that is not representative of the feline patient at large because most of my elderly feline patients have five or six diseases if not more but for the purposes of showing it scientifically it is important that they just had chronic kidney disease. So we screened a lot of cats and those 11 cats managed to make it through the study. Luckily the drug is so effective that we do get a significant effect even just with those 11 cats. What we saw was that there was a significant increase in appetite and this is coming from the daily scoring that the owners did; 91% of the cats had a weight gain when they were on the drug during the trial. Actually a lot of them lost weight during the placebo period interestingly enough. Even if they were on mirtazapine in the first part of their trial and then they went to placebo they then promptly lost the weight they had just gained; so, it kind of gives you an idea of how well they are doing in terms of keeping up their appetite and their nutrition. Also about, all of the cats who had a suboptimal body score increased their body score. Those that already seemed okay with their body score did not have a change. The other thing that we were happy to show was that there was a significant decrease in vomiting and this was not something that has ever been shown for this drug before in a veterinary species. So basically what we saw was that those cats on mirtazapine vomited much less and this cat right here, the owner was ecstatic. This cat was pretty much an every other day vomiter and it did not vomit a single time in the time that it was on mirtazapine. The owner then reported back to me later once the cat was done with the study she went back to the normal vomiting that she had.

This also has an effect, every time they vomit they lose fluid right and they feel poorly that day. That is another reason why we continued to look at other anti-vomiting medications to help out these cats because it will help with their water balance and their overall nutrition if they are not so nauseous from their disease and vomiting all the time. So that really gave us some other major important information about this particular drug.

Conclusions from mirtazapine; we now know that we have shown that mirtazapine is an effective appetite stimulant in cats and those lower doses are as effective as higher doses and have much less side effects so we always recommend starting out with that lower dose. Some cats even need less and that is when we start to get into compounding because it is not really available in a feline friendly tablet. We know that renal disease delays the clearance of mirtazapine and so we then go to going every other day with the medication as opposed to daily which is the way we use it in normal cats. We also were able to show that administration of mirtazapine in chronic kidney disease cats results in increased appetite and it results in weight gain. Although we did not continue this study out for more than three weeks it is our clinical experience that this is very, very helpful for cats that are in the end stages of their disease. My poster child for this disease is a little kitty named Chloe who lived more than a year with a creatinine of 8 before she finally passed away in her sleep and the owners were ecstatic that that was her outcome. To keep her going with a creatinine of 8, they called it meowtazapine. I keep on telling the companies that if someone ever makes this as a feline tablet they should call it meowtazapine. They really believed this is what kept her going. She kept on eating and she was bright and perky and had a good quality of life even though her creatinine was 8 for that period of time. The only

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other thing that we do need to watch out for is that there is an idiosyncratic liver enzyme elevation that can happen and oddly enough we had one cat in the study have this happen even though we use this drug like candy at CSU and I have never seen it in a clinical patient. I was happy to document it for the purposes of the study but it is actually really rare and it happens in people as well. So if you do see this liver enzyme elevation our recommendation is that is probably not a good drug for that particular cat. Just bear that in mind that we should recheck an ALT after starting this drug in those chronic kidney disease cats.

With that I would like to acknowledge all of the help that I have had. This is obviously not just me doing these things in terms of our research programs at Colorado State. Dr. Tracey Webb and Dr. Steve Dow in the stem cell project are the other important members of the Centre for Immune Regenerative Medicine. We basically started this centre based on the feline stem cell program and now it has blossomed quite nicely. The Winn Feline Foundation like I said was absolutely instrumental in starting this program for us and helping out and then since then we have been able to attract other donations and other support financially to keep the program moving along. Dr. Dan Gustafson is our pharmacologist and has helped me with all the pharmacokinetic information and the modeling for all the mirtazapine work. We do have a pharmacology core at Colorado State so that is really nice. Then Dr. Kathy Lunn who we had at Colorado and has since moved on to North Carolina was really instrumental, when I was a resident we were doing this work together and we were the ones that were just determined to show that mirtazapine was great. The Winn Feline Foundation funded our study that covered all of the research that I talked about there.

With that, thank you so much and I understand there will be little cards for questions and we will answer those later.

Thank you.

(End of Session)

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