Elise Hoover: Start with our first presenter, Dr. Abbott.

Molly Abbott: Hi. So, I'm Molly. I'm based in Oxford from the University of Oxford. So, I'm in the UK at the moment. So, my presentation is about a case that I saw as part of the medical team. Within the UK, I have a patient with polycystic kidney disease. It's also a dominant polycystic kidney disease, but I'll just refer to it from now on as PKD. Unfortunately, this patient suffered several sorts of hospital admissions and severe infections from this. And so, it led me to kind of do a literature review and a discussion with the medical team looking after him about what sort of options he had for the management.

So, just a background, as I'm sure everyone's aware, PKD is the most common inherited renal disease, but there still isn't a cure, and the surgical management options are quite floored by their complexities and the complications from the evidence base for building management plans is quite sparse, and around 50% of PKD patients progress to end-stage kidney disease by age 60.

The underlying cause of the disease isn't well understood at all, it's just known that renal function deteriorates as the cysts form and increase in number and size in early adulthood. So, since there's no cure the management surrounded by thinking about limiting symptoms, complications, and delaying treatment for delaying the requirement for dialysis.

So, my patient of a 67-year-old man who is called Mr. X, and he presented with a fever, some cold sweats, and feeling quite unwell. And so, he had known polycystic kidney disease. So, he monitored his inflammatory markers, so, his CRP on his phone app that he was able to access his blood test results, which system they use here, I'm not sure about in the US. So, he'd had two similar hospital admissions within the same year with a similar presentation.

So, this just shows his first hospital admission was for obstructive neuropathy. So, his ureters were obstructed due to his kidney disease. And this was two months before, and he presented with right-sided flank pain.

And they fitted a stent to deal with this. Three weeks later, he was then admitted again with the same presentation, and he had sepsis from the same problem.

So, they changed the stent again. So, that's the yellow and then the green on this, the pink was this presentation that I saw him for. So, again, as he presented his kidney function declined again, with his eGFR, so, his kidney function, filtration, reducing and his grastim injection.

So, upon this admission, the relevant findings for this case were, he had severe abdominal distension. It's very firm, it was difficult for him to walk around because of the size of his stomach. He had an umbilical hernia, and he actually had separation of his stomach muscles from the severe force that was inside his stomach, the pressure, he had all the observations as well fit in with him having a severe sort of sepsis infection. So, just to review, and give a clearer picture of the whole case, most of the medications he was on was actually related to the complications of his condition. So, things like the increased clot risk. He had really bad acid reflux from a hiatus hernia that he had from the pressure inside his stomach, and he had reflux disease, many years on tamsulosin for the stents symptoms he had, since his diagnosis, two of his children had been diagnosed and sorry, one of his children had been diagnosed and two of his sister's children had been diagnosed as well as his sister.

And prior to his diagnosis, he really enjoyed long-distance cycling but he was too fatigued and breathless now to be able to do this. So, there was some imaging done in the hospital and it was confirmed that there's no other source seen for the infection, except obstruction of his ureters. Again, due to the size of the cysts on his kidneys and his liver. So, for this admission, there was a significant increase with the management he had.

You can see the orange marker is the increase in renal function. So, what the doctors actually did was they took out the stent and they put in a double stent. So, they laid two cents next to each other within the ureters which they admitted was done. and sort of out of desperation because this was the third

time this had happened and after the operation, he was comfortable and yes functioning greased.

So, I had we there was a lot of discussion around what to do in terms of the management and all this sort of decision delay the two stents came after discussion with the patient as well and considering his options, so, I just had a look into the literature for the different options available. So, stenting is the main treatment for obstructive neuropathy, it relieves the back pressure on the kidneys.

And although it's minimally invasive, there is still a risk of bleeding and infection. And importantly, 80% of the patients that have this have a stent, end up with permanent discomfort or pain when they're urinating, as well as increased frequency and urination for them, so, it's not a pleasant thing often to have. And often the symptoms aren't lived by a medication. A study found that generally, you get better improvement with stenting if you have a better baseline renal function and you have smaller kidneys, so, this doesn't actually lend to us to PKD.

Anyway, also, stenting isn't curative. And as we've seen, in this case, the cause of the obstruction remains, and compression often really happens. An alternate option is to remove the problematic kidney. The perfusion is considered in about 20% of patients with end-stage kidney disease, especially those who have infected cysts. This obviously decreases the abdominal mass and for Mr. X, like he had his symptoms of pain, infection, and acid reflux, he's very short of breath on exertion, so, this would relieve a lot of this for him.

However, nephrectomy, patients need long-term dialysis, and we can't forget to consider that sort of time commitment and reduced quality of life that that involves. Also considering the scale of a nephrectomy operation, you have to think about the risk for general anesthesia. And often patients with PKD have hypotension and very aneurysm risk, potentially, which you have to think about and in the case of Mr. X, he had quite a lot of cardiovascular risks because of his BMI.

So, because of his size related to his stomach. So, then we discussed about possibly draining some of the cysts. Often, so, I reviewed a systematic review that compared antimicrobial, so, antibiotics and also surgical treatments that look at whether system infections were better managed by either. And in general, it sorts of showed that possibly long-term prophylactic antibiotics were advantageous because often when you just treat the treat with antibiotics afterward, that's not dealing with any recurrences of the infection, but obviously this also isn't curative.

Considering cysts drainage. Sclerotherapy, which is a method that uses needles to aspirate the cysts is an option. Catheter drainage, however, was found to be better for larger cysts, which is more relevant for PKD. However, this obviously introduces the risk of infection if you're puncturing cysts inside the stomach. So, this just sort of highlights the fact that all of the management options do come with significant complications.

And also, there was a real lack of evidence when looking into this study that looked from 1948 to 2014. Still only found 85 patients. So, discussing kidney transplant, there's obviously a lot of psychological stress around waiting for a donor, but it removes the option of having to have long-term dialysis with just nephrectomy alone. Some studies, interestingly, I found had shown that a native kidney size can actually reduce them the kidney will regress if you put in a new kidney, which then removes some of the complications considering of having an effect to me.

Alternatively, some studies show that people who can't have an effect to meet could have an arterial embolization you remove the arteries that supply that kidney essentially. And that can create some abdominal space and relieve some of the pressure symptoms that Mr. X had. So, I had quite an interesting discussion with the patient as well about how his family had polycystic kidney disease.

How come he his parents, he didn't know if his parents having it. And he was the one who raised the flag and his family that he had it and they all got tested. So, he actually explained that he had quite a psychological impact from this

and he'd seen his general practitioner for over 10 years, explaining that he was really concerned about his abdominal intersection, but the GP just kept telling him to lose weight and said that he should seek counseling for his health anxiety.

And it wasn't actually found that he had this disease until we had this emergency presentation in the EDE. So, I thought it'd be interesting to research whether hadn't been diagnosed earlier would have would make a difference because I had this discussion with him, and he asked me these questions. There are some trials that look at molecular therapies to target cyst growth.

And they show that if you target it, well, some studies have shown that you have less mutations when you are a child compared to an adult, which then thinks you'd have less cysts, and therefore, it could be beneficial. However, most of these studies that look at the molecular therapies for cyst growth are sort of in their infancy. And it's only preliminary data. However, if there are larger sort of randomized control trials, and some of the ones which have been shown to reduce kidney volume, could be bought into routine practice, potentially, which would certainly deal with a lot of these issues that we've discussed.

I think finally, just we thought about the impact on family planning. Mr. X hadn't been aware at all. And he said, you know, maybe people would like to be tested as like a pre-implantation. But this is quite contentious in the literature between doctors. But I thought it was something that was important to raise. So, these are my conclusions. And if anyone has any questions, please let me know.

Elise Hoover: Fantastic, thank you, Dr. Abbott. You know, I'd like to know, for cases like Dr. X, what, Mr. X, what can we do to collect more data or do more research on this kind of topic.

Molly Abbott: So, I think that was something that I found when looking into the literature is really difficult, because a lot of studies actually exclude PKD patients when

they're investigating some of these managements for kidney disease or for kidney problems because they will see it as a confounder. But obviously, that means then that a lot of these studies that even I looked at, I had to say, well, you've got your extrapolating the data to even assume that this would work for PKD patients.

So, I think reducing this as exclusion criteria, and just having them as a subgroup within studies would be one option. And also, I think, just encouraging PKD patients when they feel comfortable to, to join on to sort of data collection. I noticed at the beginning that there was a there was PKD registry. And things like this, obviously, really, really beneficial because it is a rare disease, it would be very useful to be able to collect any data on the patients that are willing to give it.

Elise Hoover: Thank you had one other question if you could just comment a little more on kidney reduction versus an effect to me kind of what you saw in the literature as far as when to recommend either?

Molly Abbott: So, I think it seemed to come across the effect to me, and, if possible, relieves a lot more symptoms for people and has better, better understanding in the literature. But also, surgeons are more comfortable with these kinds of procedures, because they're more common than doing the things like arterial embolization to try and reduce the size of the kidney. These aren't nearly as common, or sort of well-practiced. In general, the reason I looked into kidney reduction is that for a lot of patients who have PKD the general anesthesia risk of being put to sleep for such a big operation means that they're not considered, and when I spoke to the doctors that I was working with, for this patient, they said that they didn't think that he would it would be safe for him to have a nephrectomy.

Which is why I just thought, well, I'd have a look at what the other options were. So, I think in general nephrectomy, if possible, is more preferred was the general sort of impression I got, however, unfortunately for a lot of PKD patients, that's not considered an option.

Elise Hoover: Thank you. Dr. Abbott. And of course, we at the PKD foundation want to encourage everyone to speak to your doctor about your own individual care. But it's really interesting to see a case study like that. Thank you all right, let's move on to Dr. Bland.

Bland Sarah J: All right, I'm going to share my screen here. Oh, it's actually not giving me the option to.

Elise Hoover: Try now.

Bland Sarah J: Okay still not allowing me to hear we go. All right. Okay, can you guys see my screen?

Elise Hoover: Yes, we can.

Bland Sarah J: Okay. Sorry, my PowerPoint just froze Okay, is it can you see my mouse on here? Or did it move for you guys to the next slide?

Elise Hoover: Yes, just move to the next slide. Back on the paddle page down.

Bland Sarah J: Okay. All right, I think we got it. Okay. Well, thank you everyone for being here today. My name is Sarah Bland and I'm a postdoctoral researcher at the University of Oklahoma Health Sciences Senator, I have no disclosures to make today. So, today I'll be talking to you about my project that's looking at a population of trem2 positive kidney resident macrophages that are associated with slowly progressing cystic kidney disease.

So, cystic kidney diseases are multi-systemic disorders and can have several different ideologies. There are many types of cystic kidney disease. These include ADPKD, ARPKD, Meckel-Gruber syndrome, and many others. They affect approximately one in 500 people and lead to cysts not only just in the kidney, but also in the liver, and eventually lead to end-stage kidney disease and liver fibrosis patients.

Currently, there are limited treatment options available including tolvaptan and renal replacement therapy. Now, while these diseases have several

different ideologies, one thing they all share is that all of these diseases are what are called ciliopathies. So, this means that they're caused by mutations in genes that somehow affect the cellular cilia or ciliary function. So, primary cilia in the kidney are these tiny little sensory organelles that are found on the apical surface of epithelial cells lining the nephron and the collecting duct. We still don't fully understand how mutations in these genes lead to cystic kidney disease.

But one likely contributing factor in the progression of the disease is the immune cells that are present in the kidney. Now in the cystic kidney, there are a number of different immune cells present, including T cells, neutrophils, monocytes, and then the cells that lab is most interested in the kidney resident macrophages. So, Kidney resident macrophages have long been thought to be involved in cystic kidney disease.

They're believed to promote cystic epithelial cell proliferation, thereby driving cystic expansion. They're also believed to play a role in extracellular matrix deposition, thereby promoting fibrosis in the kidney. And then lastly are tissue-resident cells, they're kind of in a great position to affect both the infiltration and activation of other types of immune cells coming in.

So, kidney resident macrophages are there for thought primarily to drive progression of disease and thus be detrimental in the cystic kidney microenvironment. Now, we know in humans with PKD. During the first two decades of life, kidney function is on average, normal and cyst progression begins sometime in the second decade, patients will typically not reach end-stage kidney disease until somewhere between the fifth to the ninth decade of life.

So, in humans, this is a really slowly progressing gradual disease. Now, many of the studies implicating macrophages in disease progression are done using animal models with rapidly progressing cystic disease. So, if we look at the table I have shown here I've listed a couple of the different rapid disease models that we use in animals. Now at the top here you see the CPK animal

model, this model would be equivalent to looking at the entire course of the disease and a human between the ages of zero to five months.

So, these rapid models are not necessarily homologous to how the disease progresses and presents and progresses in humans. There are slower progressing disease models, however, such as the Cre IFT88 model shown down here at the bottom and then the PKD1 RC model that progress much more slowly over the course of the animal model's life and are a bit more representative of what the disease typically looks like in human patients.

So, we wondered whether the macrophages that are present in these slower progressing disease models would have the same function as those that are present in the more rapidly progressing disease models. So, today, I'm going to be going over data from two different models, a rapid model, the Cre IFT88 IR model that we use in our lab, and then the Cre IFT88 aged model, which is a slower progressing disease model.

For these experiments, we began by inducing cystic disease in our mice by tamoxifen administration. So, in one group of mice on the bottom half of this graph, three weeks after tamoxifen injection, they're given an ischemia-reperfusion injury to the kidney or IR injury. Two months later, we collect the kidney tissue from these mice, and we end up with three different groups. So, we have our rapidly progressing cystic group.

And then we have two rapid controls. On the other group of mice after tamoxifen injection, we let these mice just age and that let the disease progress for approximately seven months before our tissues are collected. And we end up with two groups of mice from this group of animals, we have our slowly progressing cystic group, and then we have our slow-aged control. Now after tissues are collected, we sort out the cell groups that we're interested in, and then we submit them for single-cell RNA sequencing.

Now after sequencing and processing the data, we use a program called Seurat to generate what are called UMAP projections of our immune cell data. Now, what a UMAP does is it visually shows you the different groups

we call clusters that are present in your data. And each cluster is shown in a different color. Now, the clusters that I'm showing here are separated based on their expression of certain genes.

So, in the bottom panels, the UMAP is overlaid with a heat map to show expression level of the common markers we use to kind of delineate or define our clusters. So, in the heat maps, the more pink color there is means the higher expression level of the gene that's referenced, whereas blue means less expression of the gene referenced. So, in our first panel here, where it says C1QA, this is a very common macrophage resident macrophage marker, so, we know that the clusters that have this hot pink expression are going to be our kidney resident macrophage clusters.

So, after generating our UMAP for our five experimental groups, we noticed that there is one cluster that's much more abundant and are slowly progressing disease model. And this is cluster four that I've circled here in red. And this is a kidney resident macrophage cluster that we found was defined by trem2 expression. Trem2 is, is a gene that encodes for the protein triggering receptor expressed on myeloid cells. Now, while it looks more abundant in these UMAPS, we wanted to go ahead and quantify the number of cells as a percentage of all of the immune cells that we sequenced.

And as you can see, we do see a significant enrichment of this term to cluster in our slow disease model when compared with the rapid disease model. So, this data was from our RNA sequencing as I mentioned, our next step was to verify these findings by a process called flow cytometry. Now when we do flow cytometry, we use fluorescently labeled antibodies to mark our cells and determine what cell types are present. Unfortunately, trem2 antibodies have historically not worked very well at all. And surface trem2 is also very commonly cleaved by proteases, meaning that it can be difficult to identify these trem2 positive cells by flow cytometry. So, we instead use what's called a surrogate marker, which is simply another gene that's highly expressed in our cluster, but for which we do have a reliable antibody to define it. So, in this case, we're using CD206 as our surrogate marker.

And you can see by the heat maps that trem2 and the CD206, encoded by the Mrc1 gene have very similar expression patterns in our data set. So, using our surrogate marker, we analyzed kidney immune cells from each of our five groups by flow cytometry. So, shown here are some representative flow plots from all five groups with the macrophage marker F4/80, shown on the x-axis, and then our surrogate marker shown on the Y-axis.

And so, the top box in these plots is going to be representing our trem2 positive macrophages that are present. And as you can see, in our slow model, we have a much higher amount of these cells in that top box in the graph, as compared to both a rapid model and then all of our control groups. So, we quantified this flow data as well shown here in this graph, and we saw that there was a significant increase in the amount of these trem2 positive cells as a percentage of our live single cells.

And we also have some preliminary data comparing this slow disease model to other rapid disease models. And while we have low N numbers, currently, we are still seeing on average more of these macrophages in our slow progressing model compared with all of our rapid progressing disease models. So, what I've showed you today is that while the two types of disease models we use in animals, these rapidly progressing and slowly progressing, they share many similar characteristics. But despite this, the macrophages that are present in these two disease models appear to be comprised of different populations.

So, for our future directions with this project, we first want to see whether this trend that we're seeing of more trem2 macrophages in our slow models holds true for other types of disease models. So, we have a number of other rapid cystic models we'll be comparing and then other slow cystic disease models we'll be comparing. And then we want to see whether trem2 the chain is required for this population of macrophages.

And what happens to the disease progression if we delete this gene, or if we get rid of it? So, with that, I will take any questions. And I'd like to acknowledge and thank my lab, they're always great on our long experiment

days, the research animals, and our funding that makes this possible. And then the PKD Foundation and the PKD connect organizers for inviting me to talk and putting this whole thing together.

Elise Hoover: Fantastic. Thank you, Dr. Bland. If I didn't start with a question. So, you mentioned that when you're making these mouse models, you intentionally induced an injury on the kidney and some of them, could you talk about why you do that?

Bland Sarah J: So, when you delete the cilia gene, so, when we give the tamoxifen to our mouse model, it causes deletion of a cilia-related gene. And when you induce injury in those animals following the deletion of the cilia-related gene, it leads to a very rapidly progressing cystic disease. If you delete the cilia and just let them go like, we do with our age, they still will get the cystic disease, it just takes a lot longer time so.

Elise Hoover: Make sense. And then in cystic disease, these trem2 macrophages, could you just clarify these beneficial or detrimental to disease progression?

Bland Sarah J: So, really good question. And we're not sure yet. So, in another slowly progressing disease, Alzheimer's disease, these trem2 macrophages are beneficial. So, they help restrict the expansion of the plaques and they improve cognitive function when they're present. However, in obesity, and cancer, trem2 macrophages are detrimental, so, they're bad. So, we're really not quite sure how it's going to go in our future directions.

We think that because PKD is slowly progressing similar to Alzheimer's, they might be beneficial. But we're not we're not sure we have some animal models currently in the works. So, we're really excited to see, see what it does.

Elise Hoover: And, you know, once you get through these experiments in mice, you know, what's it looks like for human patients? When can they expect to see maybe something translated to clinical trials?

Bland Sarah J: So, the exciting thing is, if these macrophages are beneficial and disease, there is already an antibody in clinical trials for humans for Alzheimer's, so, that stimulates trem2 so, it activates these trem2 macrophages. So, if these macrophages are beneficial, there is a potential treatment kind of already in the works that could potentially be used in patients. That's again, assuming these macrophages are beneficial.

Now, if they're not if they're detrimental, we could still kind of use that same plan of attack. So, another type of antibody to get rid of these, targets these macrophages, specifically, and that could also be used later on clinically for patients.

Elise Hoover: All right. Well, we look forward to seeing the results of your work. Thank you so much. Are there any other questions for Dr. Bland, feel free to type them in and we'll put them in the end if we can? All right, so, now our last presenter, Dr. DeRossi.

Charles DeRossi: Hi, everyone. Okay, so, can you see my screen?

Elise Hoover: Yes.

Charles DeRossi: Okay. Hi, everyone. So, I have nothing to disclose, we're not going to. So, let me introduce myself first. My name is Charles De Rossi. I'm an assistant professor in the Department of Pediatrics at the Icahn School of Medicine at Mount Sinai in New York. I got my Ph.D. in Germany at the University of Heidelberg. And then I came to Mount Sinai. I did my postdoctoral training. I subsequently stayed on as an instructor and then was promoted to Assistant Professor last year. So, my background is not in PKD but it's more in glycosylation and studying the metabolism and how they contribute to development and disease. By just this year, I was awarded the PKD foundation research grant and a young investigator award.

So, now I'm on testing what I've learned using mannose, which is a simple sugar as an anti-fibrotic therapy and seeing if it can be used also to treat the liver issues that develop in patients with autosomal recessive polycystic

kidney disease. So, first let me give you a background on what fibrosis is. So, fibrosis is just its excessive deposition of extracellular matrix into the interstitial space in the liver.

So, this is usually in response to injury. Whenever you have an injury or liver injury such as with a hepatitis infection and autoimmune disorder or metabolic disorder. What happens is specialized cells and the liver hepatic stellate cell becomes activated and initiate a program of proliferation, contractility, and deposition of collagen to try to combat whatever the injuries happening.

The problem is when you have a chronic liver injury, it's excessively doing this and the liver will become stiffer as if it's progressive. Eventually, you'll develop cirrhosis and require liver transplantation, this is generally **cirrhosis** liver fibrosis. Currently, the only remedy to address this is to treat the primary disease by there's an active effort to develop anti-fibrotic therapies usually targeting deactivated hepatic stellate cell and wild donor approved yet there are some that are in clinical trials.

So, in our hands, we are using a simple sugar mannose to try to develop as an anti-fibrotic therapy. And I hope as the talk goes on, you will be convinced of this. So, how does this connect to autosomal recessive polycystic kidney disease, so, I'm not going to give such a huge background on ARPKD, because I'm not going to really talk about it today. The only thing I want to liken it to is how it may fit together with like a constellation.

So, quite a constellation is the addition of sugars to proteins. And so, PKHD1 is the gene that is involved with a majority of cases of ARPKD, and this codes for a protein called fiber system, which is a highly glycosylation, glycosylated protein. So, this already connects ARPKD at least in the liver, to well in general to a constellation.

So, really I want to focus on the liver disease that develops in ARPKD and what you have here as you develop a type of fibrosis called Congenital hepatic fibrosis and bile duct ligation due to ductile plate malformations and

you can see that here in this ARPKD liver and this arises because of abnormal development of the ductile plate in the developing liver that normally would produce a biliary system within the liver but instead, they produced by dilated bile ducts and there could be downstream problems with this.

So, how does this again connect to like a constellation in general, so, we are using, what I do work on in recent years is focusing on an enzyme called mannose phosphate isomerase. So, this is a central carbon metabolic enzyme that catalyzes the conversion of mannose six phosphate and fructose six phosphate, this goes into this is an intermediate the glycolysis and mannose six phosphate is typically used by constellation because there's so much more fucose six phosphate in the cell, the mannose six phosphate generally the enzymatic reaction goes in this direction and goes towards producing like constantly proteins.

However, when you have a mutation in MPI, it could restrict the flow in this direction. And so, you have restricted glycosylation on the downstream proteins. The remarkable thing would related to ARPKD is that these patients also develop congenital hepatic fibrosis with ductile plate information. So, this suggests that there is a glycosylation component that could be contributing to developing this disorder.

The remarkable thing about MPI CBG is that it's actually treatable with mannose so, if you give oral supplements managed, you can just bypass the biochemical defect and correctly hyperglide constellation here you're seeing serum transferrin which is used as a marker for correctly glycosylation. You can see MPI CDG patients that are untreated they have a reduced level of this, but it's corrected when you get mannose.

Also, serum for enzymes is corrected here and shown anticoagulative therapy and albumin. But most remarkable is the fibrosis in the liver here measured using a technique called fiber standard which measures liver stiffness, you can see that when two patients are put on mannose it reduces the liver stiffness. So, this supports that mannose can be used in the context of MPI CDG as an anti-fibrotic.

So, we wanted to model this in the lab. And so, what we did was we use a zebrafish model for MPI CDG. And you might be thinking wow, zebrafish why would you use that? Remarkably, the zebrafish's liver is strikingly similar to human livers. And it's, it actually acts as a great model to study human liver disorders. So, what we do is we using what's called a morpholino oligonucleotide, and we deplete the RNA coating for MPI.

And we can see that in these animals with the MPI morpholino, they have a problem with that liver. Here you can see because it's lit up in white, they have reduced oscillation in these livers. And most notably is that they have five days post fertilization. So, this is at the larvae stage, they have increased the size of their ability and restructures. Because the morpholinos don't stick around too long, we also developed stable genetic mutants of MPI and in the heterozygotes, in the adult zebrafish, so, this is about 18 months, you can see that the fibrosis that they develop is remarkably similar to what looks like as congenital hepatic fibrosis with ductal platelet inflammation. And finally, if we look at the expression of fibrotic markers within the livers, we see that in these morphant livers, we see an increase in these fibrotic markers. So, the next question is okay, well, if we give them mannose, can we alleviate this fibrosis and indeed, we see that when we feed the morphogens mannose they do recover.

And if you look down here, you also see a decrease in expression of the fibrotic markers. So, the next question we wondered was okay, is this something specific to when you complete MPI? Or can this be possibly used generally as an anti-fibrotic?

So, look at this, we first stuck with zebrafish, and we induced fibrosis by giving them 2% ethanol in the water and with or without mannose, and indeed, we saw that in the ethanol plus mannose group compared to the ethanol alone group, we saw a reduction in the expression of fibrotic markers. So, this sort of suggested that okay, this is more general than simply when you have an MPI deficiency, it may be more generally used as a fibrotic marker.

So, we wanted to move away from fish then and see with this work in a mammalian system, because ultimately, we would want to use it in human patients with fibrosis. And so, we use a carbon tetrachloride liver, toxic model, and CCL4 liver toxin. And after four weeks of injecting CCI4 in the peritoneum, they develop liver fibrosis, and we will put them either on water or normal water or with mannose in water.

And indeed, you can see what the CCL4 alone, would develop this bridging fibrosis we can see here, but when we include 5%, or 20%, Nanos in their drinking water, the fibrosis was remarkably reduced. And we could stay for this using collagen. Add again, you can see the red staining is increased with the CCL4, but then when you add 20% mannose, it's substantially reduced, and that's quantified over here.

So, it looks like mannose in general is sort about acting as an anti-fibrotic. So, recall in the beginning, when I said that the hepatic stellate cells, are the main driver of liver fibrosis, so we wanted to know is mannose acting at the level of the hepatic stellate cell. And indeed, what we found was that when these are two different hepatic stellar human hepatic stellate cell lines where we cultured them in culture, we could see that just simply culturing them that these types of cells become activated.

And when we added mannose, these activation markers decreased. And this happened in a dose-dependent manner. So, it looks like mannose is acting, and this was all published in 2019. We did a much more comprehensive analysis of this. So, you can refer to the paper if you want to look more at it. But finally, what we wanted to do is what's happened to the glycosylation. So, we're currently looking at the livers in the CCL 4 model and another animal model that we're using. But when we looked in the hepatic stellate cells, are we so we did years ago liquid chromatography and mass spectrometry to look at glycosylation of global proteins. One protein that came up was the epidermal growth factor receptor. So, you can see here this is the control LX2 and in the *[Unclear]* You can see a reduction at, for example, this glycosylation site here, but when we would give mannose, it would be started by constellation abundance at that location. Interestingly,

EGFR has been tied to liver fibrosis or be going to be involved with progressive liver fibrosis.

And so, we were very happy to find this. And this is something that we're focusing on more in the future to see if this potentially could be an important player and how mannose is mediating its anti-fibrotic effects. So, just quickly in summary, so, we developed a zebrafish model for MPI deficiency that mimics MPI CBG in humans, including reduced by constellation and hepatic fibrosis with double point malformation.

Mannose mitigates the severity of fibrosis and MPI deficient zebrafish. And mannose can also mitigate fibrosis in zebrafish and mouse models independent of the MPI deficiency. And finally, mannose can reduce the fibrotic response in human HSC and restore EGFR glycosylation, MPI deficient HSC. And just very quickly, this goes on to how it ties into the PKD award that I received because now we're expanding this research into an animal model for ARPKD. So, using a PKD, PKHD1 knockout mice, we are going to treat it with or without mannose and also using EGFR inhibitor.

This actually has been published in PKHD1, deficient mice to reduce the fibrosis in these animals. And we will look downstream by clinical proteomic analysis and histology and molecular analysis. So, with that, I just wanted to thank, especially Jamie and the rest of the people in the lab. And this is my contact information. If any of you would want to reach me, and I'll be glad to have any questions.

Elise Hoover: Thank you, Dr. Rossi. We do have one question about the types of liver nodules. Can you comment on mannose in follicular nodular, hyperplasia, hyperplasia?

Charles DeRossi: Well, what do I know? I saw you mean to say effective.

Elise Hoover: Is there? Do you have any specific research on types of liver nodules with mannose or is this more general?

Charles DeRossi: So, I saw I'm not familiar with those nodules and tell you the truth. So, it is pre-neoplastic or something? Or is these just, is these cysts?

Elise Hoover: Unclear clear? I might connect you afterward with the, we **state** you ask you the question.

Charles DeRossi: Okay, so, I don't know, offhand. That answer. mannose has been shown to be somewhat beneficial for people with autoimmune disorders, that people in animal models of autoimmune disorders so such as inflammatory bowel disease because it seems to induce the expansion of regulatory T cells, which modulates the immune response, and it does this through a transforming growth factor beta mechanism? Oh, well, they're not exactly sure how that works. So, there are other disorders that this may be useful for.

Elise Hoover: What about kidney fibrosis? Is there any evidence that management have an impact on? -

Charles DeRossi: That's a great question. And actually, that's something you know, since I'm expanding into PKD. Now, that's something I would want to look at because I would think it would be effective. Yeah, so. So, this is something I'll keep an eye on as I'm running these experiments on animal models.

Elise Hoover: And then also, you know, if we're thinking about using mannose as a therapy, are there any negative side effects that you're aware of?

Charles DeRossi: So, generally, mannose is considered very safe, it's just a simple sugar, you could buy over the counter. There are some instances where patients maybe some gastrointestinal bloating have, especially if they you know, honestly, because it's not taken up very efficiently by the gut. And so, you have this. If you eat a lot of mannoses it's, it'll be a bunch of your gut and you'll have because of osmotic considerations, you'll have fluid go into your intestine.

The other thing that you have to be careful of is if you have an MPI deficiency because when you have an MPI deficiency, you can MPI is important and we moving any excess mannose and so, if you don't have or if you have if you don't have MPI, that's an embryonic lethal, you know it's not compatible with

like, if you have low activity of MPI, you may get a build-up of mannose six phosphate and this could have some detrimental effects. But generally speaking, mannose is safe, and it's already used to treat MPI CDG and used to treat urinary tract infections in the clinic.

[Audio End] [1:01:06]