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TRANSCRIPT - GR 03 01 24 "*The Sugar-coated Killer: Cryptococcosis in 2024*" John Robert Perfect, MD, from the Duke University School of Medicine

Internal Medicine Grand Rounds

- Hello, everyone! Welcome to Medicine grand rounds today, I have the pleasure of introducing Dr. John Robert Perfect.
- Dr. Perfect attended medical school at the Medical College of Ohio at Toledo. This was followed by Medical Residency at the University of Michigan, and Infectious Disease Fellowship at Duke and Duke Medical Center. In his own words, he just never left Duke, and he stayed on his faculty where he is now. The James B. Duke, distinguished professor of medicine as well as chief infectious disease.
- Dr. Perfect's research interests focus on the understanding of fungal pathogenesis through the study of cryptococcus neoformans, as well as clinical studies on the epidemiology, diagnosis, and management of invasive mycoses.
- His work focuses on cryptococcus and other mycoses from multiple angles. This includes basic science projects examining the molecular pathogenesis of cryptococcal infections, including transformation symptoms. Excuse me, gene disruption and cloning pathogenesis genes. And this work is being used to identify molecular targets for antifungal drug development. Dr. Perfect is involved in clinical trials of fungal infections, and has been awarded an and Nih sponsored mycology unit.
- He is extensively published in the field, and is a sought after lecturer. Please join me in welcoming Doctor John. Robert Perfect.
- Well, Gareth, thanks. Let me turn this on here.
- And you would think in my age that I've done this quite a few times, and I'd be kind of used to this which I am but I haven't done it for a while, and I got I have to go here, and then 2 weeks. I gotta do grand rounds at the University of Michigan. and I said, that's the first time I've done any of these talks at the places and since before Covid.
- And so you gotta get used to me a little bit here, and I'm used to talking in front of people like I used to be So Kara asked me to come to talk. It was my privilege to do that and I guess we've got about an hour so and I guess a whole mess of people also on on zoom.
- And what I'd like to do today is talk about the sugar coated killer in 2024 as she mentioned. I've been in this business for a long time. I, you will see one of my clinic notes middle way through the talk. A 78 so you can see that been around a few years. and all that time my research has been focused on fungal infections. Specifically, I started with this yeast, which is which is cryptococcus, the aforementioned. And what I'm gonna do is I see Chris coming in here is Chris Arnold. I know Chris. Chris trained with stuff like that. And morning report is great to see Chris, and he's such an energetic guy with so much energy and so much insights is prudence for me to listen. So when I asked my care to do this, I said, let me just go and try to talk again on cryptococcus, the server-coded killer.

- You can see up there. I'll get it as far as I can. I'll keep watching fine clock center, etc. If I mean, I have as many questions because I got a lot of stuff I kind of wouldn't want to go over. But there is my email address and stuff that if you have other things you want me to talk about, do? Just email me.
- Okay, so let's talk about the beast. From the beginning the encapsulated yeast. and, as you'll see in a few minutes, you'll understand where I came from this by seeing a patient 78 on Valentine's day. at 110'clock at night.
- You guys laugh, and you think I'm making that up, I'll show you. Just bear with me.
- So this organism kept looking at me that night on the eighth and I was fascinated with it. You see, a encapsulated organism. It's got just a lot of tools to deal deal with the host. And and it is an organism that has dramatically change itself in the sense of the amount of disease that we see with this organism.
- Okay? So it I always felt like these tags. Whenever you're in the fungal arena. You seem like the the guy at the bar that got to get a new Pr person or something like that. You see the slides a little bit old. It's got Sars and West dial and stuff like that the cryptocus. Who cares about crypto caucus? And do we do anything about it?
- Well, this isn't an older slide from Tom chiller and group in 2,008, looking at all the sub-saharan Africa, cryptococcosis, and other infectious diseases and you can see there where cryptok is set. Oh, that's up to Number 3.
- Now there they had deaths that were ranging up to 600,000 deaths. and at that time they had more deaths from it than tuberculosis.
- Things have changed, the epidemiology has changed on that. Surely the amount of cryptococosis that we see now is less. But bear with me. I've looked at our own institution, and we we got less cryptococcus than we did in the past, but not that, much less because we create new hosts. and I'm sure, at Virginia you have a similar type thing. So crypto cutos is at home. the outside world.
- Well, I thought, I do just a quick synopsis of what I see at Duke University. From I reviewed this one time 1996 to 2,009. It's not changed a lot. But I think it's you a perception of what we we see at our place. If I don't know what your incidents is exactly, but I would contend that you, as a central nervous system path is an outside certain viruses and stuff like that.
- My suspicion is cryptococcus is your number one organism, even over bacteria. Thank heavens! Thank heavens! To vaccines!
- I just wanted some emphasis here, a little bit on this and see if I can get this?
- Oh, yeah, yeah. Okay. So there's a when we look to this one over at the important point I want to make up here is we always put crypto caucus in HIV positive transplant recipients and HIV negative non transplants. And the 3 groups that we label them into. And this group, I think, when you look at this, the important thing that we saw and look at this is this figure right here, 47 days from the time of symptoms to the diagnosis. That's never good, because again, in that HI non HIV, non-transplant it's not on the tip of your tongue that this is going to be cryptococcus, but it does. It makes an impact on the outcome of these patients, because many times they've had these symptoms for a quite long period of time the influence that I noticed over time when I did this thing, and we looked at it about 200, some patients that we saw, and I go down to your fourth bullet there flu cytosine exposure for us. Was associated with low, low overall that mortality and attributable mortality. It was important for us to get decent flu side to see and exposure, instead of having toxicity and stop the day here, day there, etc., etc. And I think that's an important issue and one, that maybe very important for the future. As we try to develop better

strategies, we just got to make sure we get these drugs exposed well enough. The other thing that I noticed over time is not that we're any better than anybody else. But I went back and looked at the idea. Guidelines we brought out and it turned out if you fall if we follow the Dsa guidelines. We didn't for everything patient they had actually had a better less mortality and less persistence of disease. So again, I think the guidelines.

- We're not bad when we brought out 2,010, and I think for you there at least the start in the flag in the sand when you do these guidelines a patient mortality again, you look up here, trivial mortality and all cause mortality. I'm not sure which is right, but I'll start with the trivial mortality HIV, positive patients in our place. It was 16% in that time period that we looked at it. Transplant. So 17%. And then there was a non age non transplant, which is always the group that has the worst outcome for us, there's 30% mortality.
- And again, that's because there's all kinds of comers here, and you know, and I know there's no worse patient with cryptocoll disease than somebody with an underlying cirrhosis.
- That's our highest. We're definitely there's Iris sitting down there in all those groups. Iris pops up and I'll come back to that in a moment.
- Now these are the crypt cockle meningitis remains high despite our therapies roll out in Africa. This is Joe Jarvis's dad, and you can see there that it was going up, up, up, up, up, and then it came back down they were now had art therapies, and they were therapy, and they continued, even more use of our therapies, but it just kinda leveled off so the incidence of HIV associated crypto disease is leveled off in the last well, and and and the last that 10 years or so now is, that's a little bit earlier. That's 2 2,014. But I would tell you that. Just finish the ambition study which is done in Botswana and and some of the other areas and Uganda and other areas. they were able to do an 800 and some patient study 3 years ago. in 2 and a half years.
- with cryptococcal disease. So the ends of the disease. It's dropping some, and the States may be dropping some and and not some a lot.
- It is still plenty of cryptococcosis throughout throughout the world.
- In our own institution. As I mentioned, we are seeing less because of HIV, although we still see patients with HIV with crypto disease. We are making new type of risk factor patients with this organism. His organism lives in southeastern part of the United States, and I'll show you in a minute that kind of shocking things and stuff like that. You think you're all infected with one strain and stuff like that you're really not. The other thing is the average cost to cryptocomenias. I put it up there, you can see going up each one from 20,000 to 80 to 100,000.
- It just keeps increasing over a year, 2 years, 3 years. So it it's costly, not just the administration, but this, the other types of things that happen. These side effects the disabilities and various things like that. So it is a costly disease process for the for the host want to bring up. We have great diagnostics. You can't do these things without some diagnostics, but pointing out there one of the great diagnostic tests of all time, not just in fungal infections. but almost every place is the lateral flow assay for cryptocuranigen.
- Although I came in here, and Carol was mentioning to me that you had a case not too long ago that it was a with mental status abnormalities. You'd done the serum management test, and it was negative.
- And yeah, the patient eventually had disseminated. Cryptos is, I think that could have been a pro zone effect and stuff. There are just so much organism there. You

had to dilute it out. But in general, these type of tests, these type of tests, these dipstick technologies, are extraordinarily good.

- There they are. I could even do on stuff like that, hey? I even did my one of my own Covid tests the other day. So wait 15 min. And guys, those things came off. And fortunately that one band didn't didn't occur so these are simple tests. They are very accurate, and they can be quantitatively done.
- I think the other thing I want to bring up is to emphasize again combination of thermal therapy, efficient killing of a sugar coated yeast from a series of studies on one of the classic studies of Jeremy Day. Here, where they took 299 patients, randomizing. The hefty fter is being flu cytosine after to be plus flu console the best regiments. the best regimen. Fewer deaths was the amphitheatre. Me and flu cytosine. So I am still a big believer in affairsme and flu cytosine as the best induction therapy for cryptococcal meningitis. It clears the Csf faster than any of the other single or or double dog regiments and early fungicidal activity doing efas which you don't do clinically. But they do in studies have shown that there is a potential impact on outcome.
- So that's the start of this. But things have dramatically changed.
- I'm one that actually likes to do quantitation of organism. No one else does. I mean, we do. And bacterial counts of the urine. We do HIV viral loads that Efa, that effective fungicidal activity. You think your labs gonna do that, doctor perfect. I'm not gonna say that we count those organisms. And in fact, there's been some studies showing it's it's tricky to do, actually, and you gotta pay attention to it. But I wish sometime we could actually do that. So we could real be very close on following the burden of organisms over our treatment time, and you're trying to deal with the East itself rather than the Antigen. The Antigen is a very difficult thing to correlate with outcome and stuff like that. So the quantitative Cse microvois labs don't like to do it. They had methodologies that were done by multiple hospitals. Yeah, some things got all over the place.
- But at the end of the day the early funding silo activity. Although, may not be easy in a clinical study, in animal studies, in single studies and stuff like that. The idea of the ability to kill this organism rapidly gives you benefit later down the line. I think to me, that's clear for all of setting the other concept I want to bring up, and I'm not going to spend a lot of time on it. We could spend a whole lecture on it from infectious diseases.
- What crypto caucus has done to us is actually reidentify the immune reconstitution syndrome, I mean, it's the Goldilocks syndrome of of of infectious disease or immunity. Not too much, not too little. You gotta get it just right.
- And we've seen both sides of this cryptococcal disease with no CD 4. Cells, no immunity organism in every orifice, etc., etc., to ones that we start treatment on. And all sudden the immune system comes back, and we end up with Iris, and there's nothing more hard than to try to decide within your own mind is that symptom complex now, because there's too much inflammation there, and Heaven forbid! I end up having to give things that cause Cryptococcal meningitis, or make cryptococcal meningitis worse, which is steroids and those are decisions that are made at the bedside. Looking all the information you have. You are yin-yang. You are in a system, you know, where you're trying to understand the immunity. And it's changing, too, and it will change. The other thing is, I just wanna make a point that. Hmm.

- Therapeutic arsenal and all kinds of things. Now, I can't keep up with all these maps and apps and drabs, and whatever they are but they eventually find me whether they're nitro f inhibitors, whether I'll choose a map, campath or liquid aids whatever. So. And then the small molecules.
- Okay, that what? What's happening with them in the immune system, the abruptives of the world and consequences for fungal infection. I don't know. I know that can't pass. Can't be good for you. It destroys all the cells that don't need to be destroyed, and dns inhibitors have always had some fungal infections associated with.
- But you know, right before our eyes, we're just in the middle of this.
- Throw these drugs out here and let's do these underlying diseases. And we started seeing cases both. Nih started seeing some big time cases of aspergillosis in the cns and we and they use a little higher doses of a a brute name for that, for treating their their be selling phones. And we started seeing some cryptococcal meningitis. This is really dramatic cases that they started one month later. They're in the unit comatose. And so that became kind of dramatic. This
- I will tell you. The story is is very, very interesting. We published on his multiple papers have been out on it any view? The scientists in the audience, and stuff like that know? Well, this should have been an easy one to figure out we knew the site. You know the pf pkt a pt. Knockouts of the Brutin timing kinase. So we got the mouse model. Just make ourselves a a a treated Brutonid treated host.
- It's not easy. It is not easy. There's still some complexity of this thing. There's actually the knockout mice are not necessarily more susceptible to fungal disease, to to at least crypto and stuff like that. So we've struggled a little bit with the mechanisms of action. Actually with Asper, Julius is actually found some defects that it causes them white blood sales or Pms that that allow that organism to get into the simple nervous system.
- So things are changing. There are plenty of the Babs and various things out there, and you're going to see your organ like Crypto. Come, across that area.
- So remember that specific targets to wholesale lineages, all to to the mad, all that definitely risk factors for for crypto. The specific immune modulators are frequently used with other immune depressants, so be ready, be careful. There there come slight sex collectivity, for infection is real.
- The rapidity of the infection in our hands can be impressive, so you must know consequences. Outstanding therapies for serious diseases. You got to have them.
- But you gotta say that. Remember, you start playing with the immune system stuff like that. Some of your other organisms are gonna pop up and cause problems. They need to pay attention.
- All right. I'm going to switch now. That's a little bit general thing for for crypto.
- the worldwide cryptococcal guidelines. What do they tell us and what they don't? 20 24, the cryptoc guidelines came out 2 weeks ago so I'm sure all of you had it on. Your computer came up and read it overnight. So and maybe just waste of my time, it's such a knowledgeable thing. But anyway, Ill thought it was an opportunity to tell you that it does exist, and sometime you can probably find it from some link although I will tell you can help.
- There it is. That's a freaking guideline that's a lot, and you're gonna read out I'll show you how many words it is in just a second So I think that the guidelines are okay. I think that they give things. And you know, there's so much there that you can find something.

- I have actually spent a lot of my time, despite these guidelines and stuff like that is, has been a lot of time on up to date.
- When I first had up to date, somebody asked me to do the chapters on the thing. I'm I'm not. I have a real big problem, which is, I have a problem saying no.
- And so I did them, and I did them in a weekend or something like that, and
- gave to them. And okay, I'm done with that thing often. Another thing. those guidelines, the up to date guidelines. Hi Kyle understood eventually that they were freaking important because many of you in the audience stuff use those all the time, including me. I actually use it. So we better make sure we get it as accurate as we can and as easy to watch. So on the one side, where the up to date, guidelines on the other side. Set th these guidelines here they're the guidelines. I want to make a shout out to Christina Chang. A lot of this stuff never happens without somebody being a really getting down and getting dirty and actually doing a lot of the work these guidelines took 4 years you say? Whoa! That's like that. Well, they work during covid and stuff like that now, and what people are doing, and they couldn't get em together. There's the group that helped write the guidelines.
- And here's even more of the people that we're doing them. But I do want to give a special thing to Christina, because if any of you been on guidelines and various things you can do certain things, and then you forget about them or leave them. There's got to be somebody actually working on the thing at home and trying to put it together. And as you saw, that is a big stack of material.
- Well, what are the guidelines. The word count in the end it's was accepted, and is published now in Lancet Infectious Diseases the workout, 5,390 words on the original thing. References were 310.
- Then we did a supplement to the guidelines. We couldn't stop. We have diarrhea of the mind and and voice, and the word count on the supplement was 19,000 words with 510 references. There were 9 tables, 3 figures. We didn't put in a set of clinical slides. So they're now endorsed by 50 to 71 medical societies, 62 countries.
- And now I'm getting all the emails about Dr. Perfect. Why did you say this, doctor perfect? You made a mistake on this and you just gotta start thinking about how you're gonna respond. All these. Honestly, not that much yet. But but I think it's important, because it puts a flag in the sand of what's like today. In 2,024 with crypto. And I'll show you a moment. Just a little thing that for me has been really, really kind of tough.
- Okay, I can't get overlooking the organism. It's such an ugly beast. but I have to put in there. So one of the things that the guidelines tried to do? Was it tried to answer a few questions in 25,000 words?
- Should we elevate lipid formulation affairs, and B. Or F. Teres, me and resource available environments. But we never did any studies with the lipid products and resource available places. But the guide, that's what we use. And so the guten guidelines actually reflect that and non-HIV, and on transplant patients, should we encourage prolonged induction time? Because that's what the old guidelines that said from 4 weeks to 2 weeks, so we do them all consistently at 2 weeks rather than some special things for non aids and non transplants.
- That's a bedside decision, but it's but it's not favored that you need 4 weeks for and and prolonged induction therapy for non-transplant non HIV patients. We put a big question mark around Gadi I a lot of the Australians like to use 4 week induction therapy for Daddy Eye but we've and surely in the aid situation they do just as well 2 weeks as 4 weeks.

- But lateral flow assays should be aggressive, rewrite, and integrated strategy for antigen detection and treatment and resource limited environments?
- The answer is, yes, it is practical and being studied, it is out there that, in fact, should be screened in certain areas where there's 3% instance, the disease. They should be screened and then worked up if they're positive, and if they and have a negative workup, they still should be on some type of preemptive type strategy. There's several those have been done now with A is all compound, and it turns out flu, not perfect. So there's at least 2 studies that are ongoing right now with combination flu calls, or emphasizing or using a high dose ambosome are the broad recommendations of 2 to 4 weeks for starting art after beginning crypto meningitis, induction, therapy in need of revision with the coats. Data it should be, and what we put in there 4 to 5 weeks. In other words, get yourself bur organisms down, and then you can start on therapies rather than do it a little bit early and maybe get a little bit of immune reconstitution. Surely in the code studies there was suggestions there was worse outcome. If you started the antiretroviral too soon. And so However, we put in there in resource available areas healthcare systems with really close follow-up.
- And there was a paper done about a year or so ago, where they actually watch very closely that patients and started therapy started our therapies at one to 2 weeks. And patients did. Okay. I think the randomized studies didn't show that those are done in Africa. But I do think we give some leeway to people that want to start their therapy earlier if they're watching these patients very, very close.
- Should Cardio, steroid, therapy, and cryptococcosis be readdressed coronavirus are not effective in initial therapy and cryptococcal meningitis. That's the Jeremy day second study, the deck study. They're just giving them right in the beginning.
- No but it is useful in iris and Pirus, which is the post inflammatory remuneration syndrome of the neck in the back. So it depends on where you're at in the disease process, and what you have to control with information should innovate or testing be encouraged. Oh, yes, they should be considered. I don't know why we can't do mit have complete great breakpoints per se.
- But they you should always at least save these eyes, because you don't know there's gonna be a relapse and if there's a relapse having that initial islet really helps you, and trying to determine whether you got a organism that's resistant and stuff, or at least looks like it's becoming resistant. So at least you save the organism. Should we consider quantity scales, measurements? I've already talked about that way too much? It does that II just think we can't do that right now. Laboratories don't want to do it. And I think it's going to be done for research purposes. Mostly. Have we enough? Do, have we done enough with increased cranial pressure recommendations?
- There's major types of emphasis in these guidelines not perfect. I think the important message there is, pay attention to increased endocrine operation early on and try to manage it and resource limited settings without flu sizing. Should the combo of. If there's been flu be elevated to primary therapy still not optimal compared to flu cytosine. But I would suspect it's better than using single drug therapy, although and then they have up there the recommendations for the lumbar punctures Gamma interferon actually is a really interesting drug and and can be successful in the management and cryptococcal disease but just never really done a kind of a prospective randomized. And I think, unfortunately, we use it in

situations where we're failing, and there we almost never study that. Well the guidelines are there. You see the ones that gave you the reference.

- They are now integrated a lot with what's seen in who Cdc guidelines up to date the Ilsa guidelines, etc., etc., just mostly up to date. There has not been a randomized or robust trial in the US.
- For cryptococcal disease. In almost 2 decades the data is coming out of Africa. Can we use resource limited data choices need to be made. The guidelines will allow that to happen.
- Here is the situation with the guidelines for induction therapy, tricky, isn't it? Start looking at the labels. A 2 recommendation, a one recommendation that are either Liposoma, B and flu cytosyn 4 times a day for 2 weeks or the single dose, which is the ambition study plus 14 days of flu, cytosine and flu.
- No, okay, we should really all be using the ambition study that just came out in 2022. Why would you? It's an a one recommendation that a randomized study doesn't have a randomized study. Gotta be careful a little bit.
- I'll come back to that in a moment as far as and nonaids non-transplant
- Organ transplant type patients we we stayed with the standard therapy that we've done is 14 days of Amazon and flu cytosine, and consider Getty eye infections. You consider 4 weeks for induction this is the consolidation phase.
- My friend David Bowware is really pressing on when you have these things, not sure what you do actually on on these, but they feel that the Flucon is all 400 milligrams is not good enough and even those some of the early studies did is Charlie Vanderworth. So they are high on the recommendation of the 800 milligrams in the Consolidation, and you do it for one year.
- Now, the important thing I wanna bring up a little bit on this is this question is an ethical question that we have to deal with a lot on this okay that's short, Pauline. Induction therapy without one great big dose of Amazon on a base of flu colosm flu cytosine. 2 studies were done on that first one was the
- Well, it was the active study, which was one week after being flu cytosine and then one week of high dose gluconisol, and then the ambition study mortality rates the acute mortality rates and 70 day mortality rates 25%. Our 10 week is 25%.
- That's probably better than it is better than what their standard regimens are. Fluoso is very poor.
- But if you start looking at this thing and you start looking closely at it. what is our mortality rates with 14 days of this and standard Pauline induction regiments for 2 weeks.
- Mortality rates that I could get in 2 studies.
- One we did. We just went out and got insurance, dad, and started looking at the mortality rates and crypto and then
- The another people did it, too. We got anywhere from 10 to 12% for so mortality rates in the United States. And these going back and look at these, this insurance data. But recently a group in Africa are in Japan. Publish their data on induction therapy with Amazon and flu cytosine mortality rate was 6%.
- Now, does that mean that short polling therapy type thing would not be just as good as as it was. We don't know.
- But 25% is where we're at on that thing, that mortality rate. That's not good enough for what we actually have today. When you look at our standard regimens of 2 weeks of Pauline. You got to do the studies?

- Why, 2 weeks seduction therapy is a resource available welfare system. Well, mortality rates are lower 25%, 10% The polypharmacy drug interactions with fluo and Flu. Cytosine can be horrendous here in our population. To patients. They all have a lot of other drugs there on, and stuff like that, and they're coming out of Africa. It's unclear that Amazon single dose, toxicity in patients with renal disease. It might be very significant. At 10 milligrams per kilogram.
- It was done in Africa, where they don't have as much renal disease. In this patient population there are no data on single dose Amazon and transplant recipients and non-HIV and non-transplant patients. So all the data that single dose Amazon is done in HIV infected patients, no comparative studies for one versus 14 days of ambulance and guess what the length of hospitalizations day.
- Do you think that you're going to get those insurance companies to leave your patient in the hospital. If you don't have an iv and I'm giving them. Amazon, they're gonna say, why, they even freaking hospital intensity of care, send them home. Send home. Go home, go home and I don't know what the answer to that would be, but I do know. The answer is in Cryptococcal meningitis. I would not send anybody home in the first 2 weeks of management. I don't care what they're getting. I've seen too many disasters.
- Treatment of pulmonary disease. I'm going to go by that.
- I'm gonna go by preemptive thing, a couple of things I do wanna mention. Then I'm gonna finish the last group special issues of cryptocom was, need prolong induction therapy 6 weeks or longer. I think that's still in that thing. Once you have breakable involvement to do pediatric patients.
- Similar as adults. Except for adjusting their dose. Of course they can tolerate the polling. So much better.
- Cryptococcus is during pregnancy, please. They're there, and you may see patients with cryptococcosis during pregnancy, whether it's HIV or not.
- Sit and look at the guidelines. Look at all the careful data that's out there. The hundreds and thousands of patients, and then you'll make some decisions on what you want to do. You are probably not going to use a flu connozone in that first trimester the incidence of toxicity, or and triagegenity is is too great.
- Virus remains an important clinical issue. Its management is imprecise but clearly corticosteroids and severe disease and pirus diseases can make these patients much better.
- And finally, I'm going to just say bottom line on this thing, and I'm I may be a little bit wrong about this. But I'll leave, and then you can yell, scream at me later in general can be treated the same way. Their nuances.
- Okay. So now I'm gonna spend the last little bit. I started with the clinical stuff. It kind of bear with me a little bit. I've been in this business for 47 years or 48 years money long before you guys were even born.
- You got to see where my love was with this thing, or hate whatever you want to call it, and the ability to do some science on it. I was absolutely attracted by this organism. Which was born on February fourteenth 1978, 110'clock at 9.
- His name or her name? No? Well, it depends. I know the sex of it. Actually, it's an alpha. But whether the alpha is bale or female, that's a whole nother store H. 99 was born you say. H. 99. Where did you get that name? H. 99. Well. my boss had 2 books where he put isolates in one isolates from animals and one from humans.
- And so I get into the book and guess it's a human islet, which is this one from that patient. and it was the 90 ninth one I put in the yeah, I think it was not. It was not

because it was Ring Grets's number, or anything like that. It was just the ninety-ninth one.

- And so it was born that day, and we called it H. 9.
- You know. Believe I you know I'm making most of this up, I think. Not, really. But I this is my writing, my writing. I think it was bad back then. It's not too much better now. But if you look at that very closely up at the top, it says, 2, 1478, and at 110'clock Pm 29 year old gentleman with Hodgkins. Disease on predison develop severe headaches. All the types of things, has a white gown, has a hypo likeia for some of the house staff and the thing at least low glucose of 30 I didn't get the protein on him. I was late at night. I don't know what's happening. Ink was positive.
- I call it a classic presentation of cryptocomingitis signs, symptoms, disease. We're going to start after. There's the beat tonight at 5 milligrams as this test dose so like one milligram the 5 min, do it shortly, and then observe, it's the old days and a hundred 50 milligrams of flu. Cytosine is a little bit heavy back. Then we do. We use a hundred.
- That's it. That's that's the org, that is H. 99. That is the organism. Now that we've studied in the laboratories and the world has studied for the last. Hmm, I have 40, 5 years or so.
- Okay, there it started right there from that note. That is me. It signed it alright. So what is H. 99. In these organisms doing these things?
- We had the opportunity to actually start to think how it thinks by understanding itself. And then they have a lot of different things going on here. They can change their forms and stuff like that. They can be crinkled, calling smooth colony, etc., etc. So they have flexibility and guess what things can happen dramatically in the lab. So this is a fairly simple thing here of you see here, this is calling, forming units over time. And this is which I'll call stud here, and this is called Wimp which is here and
- What's the difference is is the stud was one that we had in the lab that we. We grew it up after we passed a one time through the animal. and we never grew it again. We just take it out and take a little chip of the icelet, and we don't keep growing and stuff like that. The bottom part of that turned out to be and it turned out to be an isolate, and I had given to somebody that given. Then give it to somebody else to be given somebody else, and because somebody else it was being tried all over the world, I don't know. And but I came back to our lab. Whoa! That that thing I did. We have the same strain because in the animal volume see dramatically different quality forming units and the thing and
- So the answer is this paper eventually, with a lot of my friends and stuff like that we got together, and we did whole genome sequencing of both the I. And this was, but during that time there's only a couple of changes in it. But there was a shift, a intel that got into a gene called CNAG. O, 6, 7, 6, 5. I don't know well exactly what it is. All I know is, it had some impact on phenotype.
- It was a thing that what happened was that gene controls mating so they weren't very good at sex. If they had messed up, it was that had melanin. So it didn't make a lot of melaton if it was messed up and 3 is, if we actually had that Gene knocked out. The virulence of the organism was much less so. Lo and behold! Over time we pass it around a few 100 laboratories that came back. A mutation in Dell went into that gene, that unique gene. And all of sudden it did all kinds of things to do that organism. And it just shows how you have to be so careful of things that move transposons, etc., etc.

- Well, maybe things can happen fairly fast. There's both instability copy or resistance that can occur. I've had the opportunity to now sequence about 2,500 to 3,000 strains of crypto caucus, and you see the various sequencing Vn ones, Vn. Two's the Gadi eyes and stuff like that. And we're in the process of just trying to sort out who's good and who's bad?
- I'm gonna go buy this because of time and stuff like that. I don't wanna have to play. Oh, let me do a sequencing, doing all these things. They do all kinds of stuff for these. This is, did some host expression profiles. Say, what are you talking about? I think I'll be up there on Miri modeling. What's happened is we have people in in our area that look at the transcriptome of a host and can predict what kind of organism they're being infected with whether that's a virus. whether that's a bacteria, whether it's a parasite, whether it's a fungus.
- And so over time, I said, Oh, you guys could do that and see if you can tell me the difference between cryptococcus, gadi, and neophorn by just taking blood, gene expression, profiling, finding a certain set of genes. And so they did that. Example, we just captured that thing so you could see right in the blood. There's a lymphosite sitting right there. And there's the organism and stuff like that. So they would see it each other. And then they would do these type of things, and they would change dramatically. And, in fact, during all those things there's no question we could find the genes and expression profile.
- I could tell my mouse. Whether it had a funeral infection, a viral infection, a bacterial infection, and it almost could tell that it had a cryptococcus neophora infection versus the cryptococcus Gadi infection. It almost could do that.
- So this is just an example of the host that has to deal with this organism, and it deals with it uniquely that has a certain pattern away. It does. And you can use this. And eventually, I think some of this stuff will be used diagnostic. And in the future.
- I can't go by this slide of what I've spent most of my life with, which is animal models. And just to prove to you that the person oops. There we go this person right here as watch right here. It's had it for about 45 years but it's it's gone right now. So I use this type of watch. I don't know but been great for us to understand. You can take and understand the activity of Gluconisol hephoters and B setter instead of you can go into the site of infection, and you can look at the organism and what it's doing and how it's changing.
- There's a rabbit model and it is alright. Okay. Bathroom you know. If you've been in this business long enough, you always come up with certain things you regret, and as a youngster I had a time when I was given the opportunity to take a compound called Nitran which is dystat, lipid Nystat. and as it turned out the company gave us the drug paid for for us to study in the animal models.
- We didn't have great sign and signatures and stuff like that, like in the new days, and various things that's And so we did normal doses And it was a pollen. There's a nice statin and stuff. It should had activity. It had no activity. We went multiple doses. It was L 0. So we told them. They said, Okay, fine. I said, I said, I kinda like to publish this stuff. No, you don't publish that stuff. You don't have any control where it's ours. I go okay. And they went ahead in the late 1990 S. And they studied it in 50 to 60 patients with Cryptococcal meningitis in sub-saharan Africa and it failed barest shock.
- I still got somebody in my lab right now that's talking to the company and stuff like that he wants to publish. He wants to publish our style this is, tell him these model systems are not bad, and you should pay attention to them a lot. And I actually

believe that to a point that everything's a model. So you gotta understand these things. But we got really good model. And I bring this up and talk about this just a little bit, because I'm gonna show you something in a minute that I may be completely wrong about. But I'm not in doubt.

- And I think the model system suggests that we may have the best antifungal ever developed or cryptococcalasia.
- Oh, we do all kinds of things. If you've been in this business for 45 years you could bring all kinds of people in and stuff like this. And this is our nerve, Reese's stuff. This is great. In other words, we hook them up. And we just okay, you got a bunch of organisms in the summer actoid space. Why don't we just filter all those guys out?
- At least that'd be a real fast way of kind of getting rid of. Truth of the matter is, how did you even get involved with this well, company from Minnesota was trying to subarach hemorrhages, and they were doing these filter systems and stuff like that. And I was so freaking impressed that after they filled that stuff around. You start to see clear Csf from Bloody Csf, and so we get rid of the crypto. Can't we put a new filter in there and stuff like that just get rid of it because pressure could be a problem early on, as well, you know.
- And so they said, Okay, we'll get you the system set up, and this is a quick thing of doing filtration and in the rabbit. and you can see in 6 h in 6 HI can drop the counts in the Csf by a hundred fold so I could II can just. I can just filter out that organism from the Csf. Really, really fast.
- We will see whether that makes it any farther down the line they're still struggling to get off for subarachnoid hemorrhages. I'm sure crypto is way back on their list.
- Remember that kryptcock is going global. Does it? Tell us anything? I spend a lot of time on gene expression. I always like the organism to tell me what's important and what's not important.
- I've had situations where we've had gene expression that suggested a gene like Isis trade license way way up, regulated in this in this and the central nervous system with with crypto. Oh, I think that's got to really be important.
- Well, we knocked the gene out and so we had a lcl negative mute and cause disease just like the other. So you know you get those type of things. Are you really excited and boom with that? But that's what you got such tools and stuff as you can look at this. On the other side. We had trailers. You guys know what the Sam Hills trail is. It's a sugar, and it turns out that we found that the trailers mutant was extraordinarily sensitive to all kinds of stresses and stuff like that. And, in fact, we've got now the mutants and stuff like that can die so unbelievably fast. And you and animal and humans that I think it can actually be used as a vaccine. But that's another story. But we got these things from expression profiles. But remember the global arena, which is what we're dealing with. Crypto has about 7,000 of them. And for you to find the ones that you want are the ones that may have activity. It's not always gonna be the easiest thing to do. You gotta choose wisely and not poorly. And so there's always that, and we take in Csf and isolated. There are and then we made already seek libraries, and then we kind of compare them.
- And we did this. You can see a couple of them we did here, and then we went on and separate out the genes and stuff like that.
- Then we went. After that we got 40 patients out of Africa. So now I've got 3 dozen patients. But we've captured how that organism is thinking in the human host as it directly comes out of the Csf. Not throwing it on a plate and letting it grow up and

stuff like coming directly out of the and it's a big world lot of things going on there, and a lot of things that that we can learn from sequences. We all these sequences, one things about environmental and clinical isolates. We've got 1,000 clinical islets. Now, I mean environmental islets, so are they different than the human isolates. Well, it's like, I will say, there's aggressive strains and not aggressive strains.

- You have to realize I've been a duke for 47 years but I grew up 23 miles from the Horseshoe, from the Horseshoe in Columbus, Ohio. and I don't know. It comes to football. I don't look at. I'm a big Duke basketball fan. And, by the way, good luck tomorrow night, guys, I can't be there. But there's a pretty big game going on between Virginia and Duke but when it comes to football I'm in Ohio State booty, Hayes type, guy and stuff like that, and they're not equal. And so these strains are not equal. We have different types of things, got aggressive strains, non aggressive strains may put in an animal model, even though they cause disease the environmental strains. If you look at this pose, all the environmental strains are sitting up there, we put them together. Well, I'm not hurting animals very much. Most of them survive completely. So what are the tools that are different when they get into the and but when they get into the into the clinical thing are selected out, they are organisms selected out.
- And, in fact, what I wanted to say. That is, recently we've been doing sequencing of a lot of our strains coming out of clinical isolates that we had stored away and very smart.
- Actually, I think they were very. They were very lazy.
- The laboratory was very lazy, and a man very smart. So when they had a plate of organism, and they would store it away with crypto, which was great, that they did, that they would just take a swab and throw it down here, streak through the thing and throw it in some media and and freeze it away.
- Genius. as it turned out. if they would have done things like streaking it out and looking for individual colony and stuff like that. I suspect they would have thrown away a bunch of strains.
- What happens is, if you're not doing this. It looks like in crypto. You get inhaled a lot with crypto, gets into a lympho complex, and it stays there and sits there in your Highlam for the next many years. Whatever you get immune, suppress many patients. Now that we've looked at, probably upwards of 30 to 40% of patients are infected with more than one strain.
- I've seen patients that what do you mean by strain? All right, 20,000 different snps that's kind of different. I've got 20,000 different C. Neophorman, CD. Neformins. We've had CG Gadi and CD. Neophoramans in some of these samples from an individual patient.
- So it's a new concept for us to start thinking about is that one of these, we've been infected multiple times with multiple different strains. Now, I'm gonna end here. I got 1 min although I started just to hair late talking about new treatments and stuff like that. And you see, kind of the drugs that you see up here that have been looked at in our model systems and their mechanisms on mitochondria, the G. One T.
- GWT. One inhibitors. These are very interesting compounds. They are an nostal acylase compound. And I just wanted to emphasize up here that that one's po Apx, O one, which is Manageax, and that's actually been studied in clinical trials. Now for Aspergillus and Canada. But there's congeners off that, and there's a drug that you see on the right side down there. 2039. And it's amazing. It is 2 to 3 logs more active against cryptococcus.

- Then then the it's Con, another congener. This is the most active drug in the test tube that I have ever seen. You're talking low nanogram quantities. You can see, they're both in brain tissue and in lung tissue. It sets there on the right side. It's the blue, and it's very, very low. You can hardly ever get organisms out.
- This is mixed in the rabbit where you do it over 7 days. That blue, when you see right there going down, is is 2030, 2039. In fact, we've not yet seen and going through after 8 days of treatment at the tenth day of we've never seen an organism still live in in a rabbit, and he's a brain tissue or in the Csf. This is rapid killing of the organism and then I will leave you with one final thing, because you say well, and I have a perfect talk a long time, and it's pretty energetic, I guess, to a point. But you gotta leave with some craziness. And
- what the great part of being in academia for this long is the people. And it's the people you've interacted with, both locally and nationally and internationally.
- And I'm gonna give this an up to one of the students that just that it's amazing to me. He was an undergraduate student.
- perfect. I've been reading about these Ketogenic Ketogenic diets. and they use it for seizures and other types of things, and I wonder whether we could change the environment a little bit, and the Ketogenic diet! To make some of these drugs act a little bit differently.
- I go. Well, I don't know. It seems like a long shot. but I'll tell you what, you're a good guy, and I think you're worth at least 40 mice and so he got 40 mice and let me just say I was shocked. You can see it up here if you look really closely at at this right here. So this is the vehicle. This is the Ketogenic diet itself doesn't do anything is right here. It's starting to get some activity with the dosing we were using. But you got several little more kill with all and the Ketone genetic diet. So something in the combination doing this, and you see that is for both the brain and if no right side you'll see, for for the long I've done this thing multiple times. It is as consistent as it could be. And I've also done it. With Canada you look up there again, little different dosing and stuff like that, and specifically sit on that left side, but that the combination of flu causal plus plus a ketogenic diet you get increased killing in the kidney. So what I saw up there quickly. Multiple times, different site of infection, different organism that if you change the environment with this Ketogenic diet and you use Gluconisol you can get better activity than in the standard way to do it. So I don't know where we'll go with that boy. I do. I do. I do. It's and I'm bio. Okay. So we're we're clinicians. We're academics. We publish right and so that paper should come out. And then bio in about a month or so.
- But now I've got David Bullwear and his group in Africa interested in this because they're interested in new diets and cryptococcal disease and stuff like that. Where here's a diet.
- We're going right here. That looks like it can synergize with Fluosal. And if we do it just carefully, maybe they do all those quantitative counts and stuff like that. And so who knows? I come back in a year or 2 years, or maybe David comes down here, and he has this famous keto diet stuff like that use for cryptococcal meningitis potentiate. Say, he's all compound. You first heard it here by me so again. Cryptic sugar coated yeast 5 min over. It is important. It's deadly. It's mysteries are being unwrapped.
- Spent a lot of years doing this in the treatment. The goal is efficient killing of the sugar coated yeast. We want fungicidal activity, fungi targets. There are weak

spots. We can and kill that organism, and we can kill it very efficiently. And we have some new drugs, some new class of drugs that are absolutely outstanding.

- But remember, it's always the host. It's the yeast. It's always a two-way street whenever you're dealing with it.
- So this is my lab smiling lab. I'm not sure why they're smiling. But well, because I'm smiled, I don't know. But anyway, they're the ones that do the work.
- And this is my crypto team at Duke up unfortunate when you're better 46 years or 47 years. Yeah, you try to get people on your team. And
- I got Joe Hyman on my team. And that was a really big start cause he's a outstanding scientist. His lab is massive now with, has about 15 post docs or something like that so? It. It amplify all things we do, and also the collaborators we do with the outside of do because one of the things I think, for all you young people and all the people that are I would always encourage you in academia always encourage you can, is to find partners to find people outside of your areas that you can collaborate with and connect with it, matures you so much more, and gives you much, such a wider viewpoint of things. So be not afraid to collaborate and connect, if you can, with whatever you're doing, at least from my standpoint. I think the best thing I did was probably I became more well known outside of Duke than inside of Duke for a long time. And second of all was I made a lot of connections with a lot of people, and then I was able to accumulate people working my labs. Some of them are some really great scientists that have gone on to take this thing farther.
- So I'm sorry it's 107. So I'm over and I'm gonna stop right now. I am here. There are questions and stuff like that later on, and I don't know how you do this thing, but I'll I'll show you. They gotta get off. Get back to work. I just gotta drive back home to Durham. So I could stay here forever. But again I'm I'm here. If whatever you need to do as far as ask questions I will do so. Thank you.