[MUSIC PLAYING]

CHRISTOPHER B. HUGHES: I'm going to talk about machine perfusion of the liver. And what I'm specifically going to talk about is normothermic machine perfusion, which is basically we take the liver out of a donor, we put it into a machine, connect the vessels up so that we can run oxygenated blood and whatever solution we want to through that liver until we get it to where the recipient is, and then we take it off the machine and put it right in the recipient.

Doing, this instead of operate on the donor, put the liver in ice, take it to the airport, fly it on the plane, put it in the ambulance, drive it to UPMC, wait for us to get half the liver out, the old, cirrhotic liver, put the new one halfway in until we can reperfuse it, and all that time, we've got this cold ischemia going on in the liver that we don't know, really, how much damage is going to be done.

So there is a trend in liver transplantation where we're moving toward, what can we do to preserve the function of the liver? And what else can we use machines for to help deceased donor liver transplant work well so we know the liver is going to work? And we don't need this so much in living donor liver transplant because we take that liver from the room.

When Dr. Humar and I do one of these cases, it comes from the room right next door to this room, and then we start putting it in. So there's really not much ischemic time. So that's what this talk is about.

You probably all know these people, especially if you're in the transplant world. Dr. Murray did the first kidney transplant; Dr. Starzl the first liver transplant; Christian Bernard, the first heart transplant. All these guys were on the cover of *Time* magazine in life and got lots of media attention.

Dr. Hardy, who did the first lung transplant, was from Mississippi. So the first lung transplant was in Jackson. And he didn't really get much media attention when that happened, and I'm not going to go into why. Remind me at the end of this if there's time, I'll tell you the story. But he was kind of the odd guy out.

There's another guy. I call those other guys famous first. They were famous for doing something in transplantation. There's another guy, I'll call him the infamous first. Does anybody know who this? This is Dr. Robert White. He's a neurosurgeon, was a neurosurgeon. Died in about 2011.

Spent most of his career at Case Western. And if you want to find out about him, all you have to do is google "monkey head transplant." And there's some videos. They're kind of gruesome, just so you know. And he decided that he was going to figure out how to transplant a head.

And so he used rhesus monkeys. This was about 1970. Figured out how he would swap the carotid artery, and basically, he swapped heads. The animal rights activists were really bent out of shape about this, as you can imagine. He kept these monkeys alive. I think the longest one lived about two weeks.

And they could open their eyes and look around. Of course, they were quadriplegic because he didn't have any way of attaching the spinal cord. And even as you can tell in this picture, he even had to have an external apparatus to hold this being together, because the spine wasn't really connected in any way.

So I tell you this story not because this is a talk about brains or anything, and he didn't use a machine for this, but I tell you this just because last week, our office got a call asking for a quote for *The New York Times,* because they're doing an article on a paper that just came out in the scientific journal *Nature* about somebody putting a brain on a machine perfusion device to keep it alive.

So that happened last week. This was out of Yale. This is from their paper, where they took pigs that had been slaughtered for food. So they said, these pigs are dying anyway. And they took the brain four hours after the death of those animals, where the brain was just sitting there. And they put the brain on the machine and ran it for six hours with oxygenated-- it wasn't blood, it wasn't a cellular fluid, but it was oxygenated. And they found that some of the cells could be revived, even that far out.

And so the question that *The New York Times* want to know is, what does this mean? Are we trying to keep brains alive, or are we going to transplant brains and all that? No, not really. The point of their research was not to do anything as far as a brain transplant, for instance. It's how can we use machines to better study the brain?

Because unlike Dr. White, who had to put a head on a monkey and now this monkey has to go through whatever for two weeks, we don't know what pain it's experiencing. We don't know any of those things. And whatever blood tests have to be drawn, or testing, or whatever. If we could have just taken the brain and put it on a machine, you could study it however you wanted.

You could study all of these enzymes coming out. You could do biopsies, markers, all of that, and not really have a suffering animal. So that's what machines can help us do. Machines are here to help us. They're not here for us to do something to threaten the world with this brain and a machine thing, like *iRobot* movie.

So these are what real transplant machines look like. This is probably the most popular one, which is the LifePort made by Organ Recovery Systems that's used for perfusion of kidneys and is a portable device. It's used pretty frequently. The ex vivo lung perfusion machine is used to place marginal lungs on a perfusion device so that those lungs can be recruited, recruit the air spaces, see if those lungs are potentially transplantable. This is a liver on a machine device. You can see the cannula is going into the artery in the vein to circulate oxygenated blood. This is the OrganOx machine.

And there are basically, right now, three commercially available machines for perfusion of livers. The OrganOx metra is a company in the UK. There is a large multi-center trial going on right now in the United States and Europe to study livers on this machine. The TransMedics Organ Care System liver device is a machine made by the TransMedics company, which is here in the United States. And they're also running a multi-center trial, and we're just about to join them. The protocol is in the IRB.

And then the Liver Assist device is another device that was invented in the Netherlands at Groningen, which is kind of famous for devices. They were also the place where Willem Kolff, back in the '40s, invented the dialysis machine. So those are currently the three commercially available machines.

But machines are basically there for us to try to do-- I'm grouping them in three things. One, can we assess the function of a liver, like a marginal liver, one that we're not sure if it's really good to transplant or not? Can we assess the function of a liver while it's on the machine to make decisions about whether it's suitable for transplant?

Can we treat the livers while they're on the machine? Can we treat the liver to make it a better quality before it's transplanted in the recipient? And the last thing is-- I say protect-- can we protect the recipient from the potentially life-threatening post-reperfusion syndrome that happens with just about every deceased donor liver transplant for some degree?

So let's talk about assessment first. What livers would we want to assess on a machine before we transplant them? Well, we'd want to use the ones that we're most concerned aren't going to work. Those are the DCD livers, Donation After Cardiac Death. As most of you probably know, that's an increasing number of livers available now, are DCD livers.

Those are people who do not meet brain death criteria. But their family has decided to remove them from all life support because they have such a brain injury that it's not really conducive to life, but they don't meet brain death criteria. So we can't just go and operate on them with their heart beating and blood circulating and take the liver right out.

We have to wait until they're extubated go through a period of hypotension, and then asystole, and then a waiting period to make sure that they have died by cardiac death, and then we can make an incision and take the liver out. So that adds an additional "warm ischemia time," where there's damage to the liver because there's a period where it had no oxygen. CORE this year is at about 50% of the deceased donor livers available this year from CORE, our own OPO, or from DCD livers.

Older donors, donors older than 70, those livers can be used for transplant. Some of them are good, some of them are not. But in this country, about 2% of livers over the age of 70 are used for transplant. There are a lot more that are thrown away, obviously. 98% of the others are thrown away.

And then fatty livers. A liver, if it's declined by a transplant surgeon, I don't want to use this for my patient, I'm going to decline it, the usual reason is because the liver's fatty. It's the most common turn down reason for a liver.

So in 2001, a study came out by Shone, et al from Berlin, which they studied pigs. And they put a pig liver on normothermic machine perfusion. That means oxygenated blood, running through the liver for four hours, including a one-hour ischemia time, which means they did a model for donation after cardiac death. They let the liver sit for an hour, put it on the machine for four hours, re-oxygenated it, and then transplanted it.

In pig terms, that's a big deal. Because although human livers, we can go about 12 hours of ischemia, if you do four hours of cold perfusion-- putting a liver on ice, a pig liver, and then transplant it into a pig-- their survival rate is really low. So pig livers really are susceptible to ischemia.

And so what he found, what their group found, was that after they perfused and then transplanted, the livers that had been on the machine showed way less damage, as far as the liver enzymes after transplant, than did the ones that had been in cold storage. That's one of the earlier studies looking at normothermic machine perfusion.

Pretty similar findings, but a little more elaborate study, came out in 2002 with Andrew Butler and the group in the UK, who put a pig liver on the machine and kept it going for 72 hours, basically with the same thing, normothermic machine perfusion, oxygenated blood. And what they found was that the liver enzymes really don't change. So there's not a lot of damage during that time on the machine. They found that oxygen consumption continues throughout the time that it's on the machine, so the liver's working. It's using oxygen while it's on the machine.

But they did notice that the liver made bile for about 10 hours, and then it quit making bile. And we use bile as a marker for how well the liver is working. If it's producing bile, it's doing all the things it needs to be able to produce bile, so it's working. But after about 10 hours, the bile output dramatically went down and the bilirubin went up.

And they determined that, well, that's because our solution that we're using has no bile salts, no bile acids to use as substrates to create bile. So ultimately, after about 10 hours, the bile ducts will get filled with this sludge and they basically have biliary obstruction. That's just a matter of changing the perfuse aid. Modern perfuse aids contained bile salts and bile acids.

Fondevila in Barcelona did another study, also in pigs, using a DCD model with 90 minutes of cardiac arrest. Now, that's a long time. In humans, we'll take about 30 minutes of cardiac arrest. If it's longer than that, we're too concerned that there's going to be damage to the liver, that it's not going to work, or the bile ducts are going to die because the cholangiocytes are really susceptible to warm ischemia.

And so what they found was that the livers that have been put in cold storage, once those livers were transplanted, the AST dramatically went up, suggesting that there was damage to the cells. The bilirubin went up after transplant. This is quick prothrombin time. This is a measure of coagulation. Coagulation was basically out of whack in the ones that were getting cold storage.

And none of those animals, by the way, survived the full five days of the study. All of the animals that had normothermic machine perfusion did survive. And not only did they survive, their liver enzymes were basically normal. They had a little bit of peak. Bile salts, bilirubin stayed normal. Prothrombin time stayed normal.

And when they looked at cytokines, cytokine release being a measure of inflammation, they found the same thing real, high levels of cytokines in the ones that were cold stored. That's TNF, IL6, endothelin-1. All of these things are suggestive not only of hepatocellular injury, but also endothelial injury, which are the linings of the vessels.

Those things are important because if you incite an inflammatory response, which is basically what is happening-- the liver's getting transplanted, and because of this cold storage, there's this incitement of an inflammatory response that now is going to have an effect, not only on the liver, but as you're going to see, an effect on the patient that we just put that liver into. So if we can prevent all of this liver damage and all of this injury and cytokine response, inflammatory response, we're going to help not only this liver, but we're going to help the patient.

And so ultimately, what we want to know is, well, if we have a liver on this machine, what do we need to know for us to say, hey, I think this liver is going to work when we put it in a person? And so that's what another group at UK looked at, Brockman's group. This paper came out a few years ago. Actually, it's more than a few years. It's almost a decade now.

But this is also in pigs. They found that the successful liver transplants all showed good bile output. The pH was controlled by the liver when it was on the machine. The enzymes were relatively normal. Hyaluronic acid was normal. The portal pressure and the portal venous resistance, meaning the flow through the liver, was good the whole time.

Those that they put on the machine, that those things were not good, were the ones that failed. And so can we take a liver that we're not sure about, maybe a 75-year-old donor or a fatty liver donor or a DCD donor, put it on the machine. We can gauge these things. We can do lab tests. We can monitor the flows. And then make a decision, yeah, let's go ahead with the transplant. Or say, you know what? This liver is not going to work. We're going to cancel the case.

So for donation after cardiac death, the normothermic machine perfusion is good because it could reduce our overall ischemia time, because now we're just getting it on the machine and so it's a quick time that it has going from donor to the machine. We can assess the level of cellular damage, the level of endothelial injury. Same thing for older donors.

What about fatty livers? Fatty livers are a special case. And whenever we have a donor, they'll call and say, hey, this BMI is 50. Do you want to accept this liver? I don't know. If I had a biopsy, that would help me. Usually, we'll take about 30% macrosteatosis. And if it's more than that, we'll turn the liver down because we're afraid it's not going to work, and here's why.

So livers have two types of fat, basically, in them. They have macrovesicular, which is a big fat droplet within the cell. And it's so big that it squishes the nucleus out of the way. It kind plasters it against the other parts of the cell. That's macrovesicular steatosis. Microvesicular steatosis is where there's little droplets in the cell, but the cell architecture is basically OK.

And when a pathologist reads a biopsy, he'll give us levels of both. He'll say, it's 30% macro, 70% micro. Do you want to use this liver? The micro we're really not that concerned about. It really doesn't have an effect so much on transplant. But the macro will. And so some of these biopsies that we'll look at, some of them will be mild. Some will be moderate. Some will look like this, where almost the entire liver is replaced with fat.

So here's the problem it presents for us when we go to transplant someone. So this is a sinusoid. So this is the lumen of a sinusoid, and these are the hepatocytes that are lining the sinusoid. And these are the hepatocytes that have macrovesicular steatosis, big droplets that are making the cell big and pushing the nucleus out of the way.

Just by virtue of their size, those fat cells already impinge on the lumen of the sinusoid. Now, sinusoids are really small. Here, I've shown that the red cells are enough where several can go through it at once. In reality, a sinusoid is about big enough for a red cell to kind of turn on its side and slide through. So any impingement inhibits the flow.

So there's infringement on the sinusoidal lumen. Then because of that impingement, when we go to recirculate that liver, there's going to be relative ischemia damage because the flow is not as good. So all of these hepatocytes now, that are trying to get oxygen from the cells that are coming through this lumen, are not able to do so because the flow is inhibited by these big, fat cells.

And that damages their electron transport chain. When a cell gets ischemic, its electron transport chain, which is very dependent upon oxygen, gets damaged. There is leakage of electrons from the electron transport chain, which reacts with oxygen to create reactive oxygen species, which then causes Kuppfer cell, which are the white blood cells in the liver, basically, to be activated and produce cytokines, like TNF and IL6 and endothelin-1, those things we just talked about that are damaging to liver.

That causes adhesion molecules to be released because now there's damage to the endothelial cells that are running along these hepatocytes, lining these hepatocytes. And that causes platelet and leukocyte adherence in the sinusoid, and that further blocks the sinusoid, so now there's even more of ischemic damage. So you transplant a fatty liver, you get ischemic damage. The flow is bad.

All this inflammatory stuff gets started up. It makes the ischemia worse, which makes the inflammation worse. And pretty soon, this liver is going to stop functioning. The enzymes, the AST and ALT are going to be 10,000. And you transplant this person, you go look at their labs eight hours after the transplant, and you say, oh, man. This liver's not going to make it. Then you're in a pickle.

So it would be nice to know that ahead of time, if we thought this was a bad liver could. Could we put this on the machine, prevent the ischemia in the first place, and then see if we think this is going to function, or if it's going to be one of those livers where the enzymes go real high no matter what we do, and so we won't transplant?

But the machine might offer us another option for these fatty livers. Could we treat that fatty liver while it's on the machine? Because we could treat it with whatever we want. We don't have to worry about side effects of a drug, for instance.

If a liver's on the machine and we want to give it some drug that could get rid of the fat that's in the liver, that's better than giving that drug to a person, because that drug's going to go to the whole person. So we don't have to worry about side effects of nausea and diarrhea in a liver on a machine, like we would in the person. So could we treat this liver?

So let's say we have a fatty liver and we put it on the machine. Are there drugs that we could use to treat that fat? Well, if this is a hepatocyte and there's this big lipid droplet there, just as an example, there are beta adrenergic receptors, glucagon receptors, that can be triggered by drugs, like forskolin or glucagon, that can increase cyclic AMP, which activates protein kinase A, which activates cytosolic lipases, which then start to break down this big, fat droplet.

And you get free fatty acids, and those free fat fatty acids become ligands for peroxisome proliferater receptor, and liver X receptor, which increase enzymes involved in catabolism in free fatty acids. And there's even a drug that can actually activate these enzymes to cause lipolysis.

And so can we treat, even with high doses of these? And I think on the previous slide, [INAUDIBLE] showed that 30% decrease in triglycerides of the liver of rats within just three hours. We've already shown we can keep a pig liver on a machine for 72 hours. How much could we de-fat a liver before transplanting it into a person to reduce their risk that that liver is going to fail?

So this is just one of the ways that we could potentially treat a liver before we transplant. Or another is what about a hepatitis C-positive donor? We could start hepatitis C treatment on the machine before we even put the liver in. Lots of opportunities to try to find ways to use more livers to transplant people.

So we talked about assessment. We talked about treatment. What about protecting the recipient? So whenever somebody has a liver transplant, particularly from a deceased donor, and we're bringing this liver that's been in cold storage for eight hours, we've taken that person's cirrhotic liver out-- that's the dissection phase-- the blood pressure, cardiac output, everything's relatively stable. We make them an anhepatic, so we clamp off all the vessels. We get the liver out. And now we're starting to sew in the new liver, getting ready to reperfuse it.

A cold-stored liver now has all of that stuff we just talked about sitting in it. It's got TNF and IL6. It's got all these vasoconstrictors, all these liver enzymes from the cells that have died inside this liver as it's waiting to be transplanted so it can try to recover from being transplanted. And now we're going to release all of that into this recipient.

And so they get this post-perfusion syndrome. And actually, Dr. Hilmi, who is one of our anesthesiologists, has one of the seminal papers on this phenomenon. But basically, we reperfuse the liver and all these things get released. And all of a sudden, blood pressure drops. Cardiac output drops. Patient basically is tanking, depending on how severe this is.

We don't see this very severe at all in the living donor recipients because it's such a short ischemic time. In the deceased donor recipients, we see it for sure. The end result, the worst result, that we can see is that the hemodynamics get so bad during that time, and now we are trying to vasoconstrict them and trying to get their pressure up, whatever we can do, is that they develop intracardiac thrombus in the operating room. And we see that because we have a TEE that's constantly monitoring these patients during the procedure.

There's no way, really, to avoid this. So we have, actually, a protocol. It saved three patients in the last two years, where we have tPA on the anesthesia cart right by them at the time of recirculation so that if this happens-- this is a terminal event, unless it gets resolved real fast. And no matter what kind of resuscitation you try to do, it's not going to get better if there's a big thrombus in the heart.

So we can try to tPA those patients, but now we have a patient who's just been tPAed and we're in the middle of a liver transplant. And so now it's going to be a long case as we try to get dried up, but saves their lives.

So could we monitor all of these things, while the liver is on this normothermic perfusion, and basically not put the patient through the post-perfusion syndrome? Put the liver through that syndrome while it's on the machine before it goes in the patient. Because we can control this.

Get all those things out of the liver, then put it in the patient, and now we don't have to worry so much. And that's what the earliest patients that have been in the trials in Europe and Asia with the normothermic machine perfusion have shown, that the reperfusion is much gentler than it was with cold storage.

So basically, the trial that we're about to be involved in is the TransMedics trial. This is their machine. It has, basically, a disposable cartridge that is used for each liver that's put on that device. It has a wireless monitor that lets us look at artery flow, vein flow, what the oxygen saturation is. We can deliver substrates.

So any drugs, other medications that we want to put into the liver, we can. Bile salts, whatever it might be. We can measure the pressures. And we can use all of that information to decide whether or not we want to use that liver. These are the other centers that are in that trial.

And basically, we're taking donors over the age of 40, where we think the cross-clamp time is actually going to be a long period of time. We're also using DCDs and steatotic livers right now, as long as they're below 40%. I think the next trial after this one-- there's several machines going through similar processes as this.

This is trying to get the approval by the FDA. And then once the FDA approval is obtained for these devices, then we can start to do things like looking at treatments, and those kind of things, in humans, instead of relying on all these pig study data.

So right now, it's just trying to prove that the machine is as good as cold storage. It's going to be better, but we have to prove right now that it's as good to get FDA approval. So that's kind of the story of machines. So thanks.

[APPLAUSE]