Third ACTTION Scientific Workshop
Transformative Strategies-Development of Pain Therapies

June 24, 2014

A Matter of Record
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THIRD ACTTION SCIENTIFIC WORKSHOP

TRANSFORMATIVE STRATEGIES FOR THE DEVELOPMENT OF PAIN THERAPIES

Tuesday, June 24, 2014
8:35 a.m. to 4:44 p.m.

FDA White Oak Campus
White Oak Conference Center
Building 31, The Great Room
Silver Spring, Maryland

C O N T E N T S

C O N T E N T S (continued)

P R O C E E D I N G S

(8:35 a.m.)

DR. PORRECA: All right. Well, good morning, everybody, and welcome to the second day of our meeting. And it's my great pleasure to introduce our first speaker this morning. Our keynote address is going to be presented by Dr. Clive Svendsen on the topic of human pluripotent stem cells.

Just to tell you a little bit about Clive, he is the director of the Regenerative Medicine Institute at Cedars-Sinai. He's been working in the area of stems cells and regenerative medicine now for many years. He was at the University of Wisconsin for some time and founded and ran the stem cell biology program there.

He's subsequently moved to Cedars-Sinai, where he's set up and directed Regenerative Medicine Institute, which now consists of 120 people and 16 faculty, and working on stem-cell based therapies for all different types of diseases, as he's explained to me, from liver to

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brain.
So today, he is going to be presenting on the topic that I know very little about, but really offers perhaps tremendous promise for the development of pain therapies. And he's going to be talking about stem cells, GDNF, and pain. And so Clive, thank you very much for coming.
Presentation – Clive Svendsen
DR. SVENDSEN: Thanks very much, Frank, and to everybody for the invite. Despite United's best efforts, I made it here. Just a bit of advice. Never go through Chicago on United at 4:00 in the afternoon.
I actually changed the title a little bit. Yes, I work on stem cells, but I've also got a long history in GDNF and somewhat neural transplantation as well, which I thought would fit a little bit with the next speaker, Allan, who's going to talk about some of the work he's doing presumably on putting neurons in the spinal cord to control pain.
So I was asked to give a broad overview, so I'm going to do that first. And then I'm going to

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try and keep mentioning pain as I go through to get it back to pain and how the stuff we do may impact pain.
So this is the building I work in at Cedars-Sinai. Cedars-Sinai is a 1,000-bed hospital in Los Angeles that is now becoming a major medical center. We have over 600 faculty working in the sciences and a huge science program, really matching all the pinnacle efforts that we have.
The big picture, first, I'll talk about stem cells at the moment to update you. Then I'll talk about modeling diseases. And I think many of the impacts of stem cells may not be a therapy. It may actually be modeling, so modeling pain in the dish.
Then I'll talk about a new trial to treat ALS, Lou Gehrig's disease, using GDNF-secreting stem cells, which again I think has impact for pain, given the work Steven's done. Sorry I missed yesterday. But the impact of GDNF on pain may be significant, and we may be able to revisit that in the discussion, and then talk about iPS cells at the end and how we might be converting to those for

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different types of therapy.
So the big picture, if I'm going to lead you with anything, this will be the thing to remember. Up until recently, stem cells were mainly thought of as ethical issues. The embryonic stem cell era that just ended about four years ago was fraught with problems.
When I arrived in Wisconsin in 2000, the paper from Jamie Thompson had just been published, where he destroyed embryos in order to make embryonic stem cells. And this whole fervor in the field, there's been more ethics about this topic, that science has really been the driving force of media attention to the field.
This all changed when Shinya Yamanaka, who got the Nobel Prize a few years ago, discovered that we don't actually need embryonic stem cells anymore. And I'm going to really play the view here that embryonic stem cells, while important, today are probably not going to be relevant anymore for the field.
The reason is that you can take a diseased

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patient, or any patient, up to 100 years old, isolate cells from either the blood, the skin, or even the liver, any adult cell, put them in the petri dish, and grow them for a day or two, and then expose them. And I'm not going to go through all the technology.
The main factor is Oct-4. It's a transcription factor that if you just put the protein in an adult cell, any cell, it reprograms the cell. It spins it back in time through demethylation events back to a pluripotent state. So once you do that, you end up with a dish with a cell that's almost identical to an embryonic stem cell.
Now, this reprogramming of any somatic cell really took the field by surprise. Unlike STAP technology that's just been discredited recently -- those of you in the field know a new technology from Japan -- iPS technology is real, and many groups have reproduced it. In fact, there are over a thousand papers now using these induced pluripotent stem cells. And you can generate them

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from humans. So any human patient or human, you can generate these pluripotent stem cells from. So now, essentially, you can all have your own personalized embryonic stem cell line.

We use these cells for a number of different tasks. We can also engineer them. They're easy to engineer because they're pluripotent. So we can edit genes, put genes in, take genes out. In the institute, we actually grow them into skin, brain, nerve, blood, heart, kidney, muscle, lungs, stomach, liver, and pancreas.

In fact, in testing, we can actually make whole organoids from these iPS cells, so we've taken them from Crohn's patients, made them into iPS cells, skin cells from Crohn's patients, and then developed them back into whole gut organoids in the dish. So we can get a Crohn's gut in the dish and look at things like absorption across that, the tissues in that gut.

So this has really opened up the whole area of autologous stem-cell transplantation, allowing now to have matched cell types from patients'

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transplants.

At Cedars, we're really entering into this personalized medicine area where disease stratification will be important. And that means also we can generate cells from the same patients for transplants, maybe correct the mutation if there is a mutation, and generate these patients' specific lines.

So transplantation is important, but also, I'll talk about making tissues and organs in the dish means we can learn about disease mechanisms. We can actually recreate a lot of diseases now in the dish with human cells.

Finally, we can make beating cardiomyocytes from these iPS cells. So if you think about toxicity screens for drugs in big PhRMA, a lot of the problems in the drugs occur because they have problems with arrhythmia and cause arrhythmia in patients.

Here, we have shown now, and other groups have shown, you can put drugs on beating cardiomyocytes from a single patient and show

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whether that patient cardiomyocyte beating will be affected by that specific drug. So this is personalized medicine and drug screening that means you can now screen drugs with side effects on patients using their cells in a dish.

All of that is coming, so I think you have to be ready for this kind of revolution that's happening in that we can now grow tissues and organs in the dish.

This is my simple slide. The problem is, if you make these iPS cells incorrectly, you end up getting false data out of the other end, so rubbish in, rubbish out. I think the key is to make quality cells and quality lines, which is why we have an iPS core facility that qualifies the iPS production and runs it through a stringent test before releasing it out to the faculty we have.

But really, the idea of this disease in a dish, for me, it's a little bit like Avatar. You can now have your patient in a dish, essentially. Now, as we get more sophisticated -- we started in this field back in 2009, and I think it's one of

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the first papers showing we can actually do this. And this is a disease called spinal muscular atrophy, a serious disease in children. At six months, they become floppy, lose all muscle innovation, so essentially, this is a multineuron disorder.

We took these patients, made iPS cells from them, as I just described, from the skin. And then we pushed them from an iPS state to being neurons. And in fact, we can make them into specifically the multineurons that die in these children.

What we showed in this paper was that, up until about 4 to 6 weeks of differentiation, the multineurons are okay. But between 6 and 10 weeks, the neurons from the children started to die, whereas the neurons from the healthy parent lived. So this was the first model of a neurological disease in the dish, and we can play this over and over again.

Once you get iPS cells, they essentially mortally reactivate telomerase. You can grow them forever. And now you have a limitless source of

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pluripotent cells that you can do these disease-modeling tricks with. And in fact, this now we have taken on -- I won't show you the data -- into drug screening, looking for molecules, which prevent this cell death in the dish, which mimics the human condition, a very exciting technology.

We have also done this for Lou Gehrig's disease, and I'll talk about, in a moment, a more specific type, a genetic form of Lou Gehrig's. And we managed to get a disease model here where there wasn't overt cell death, but we saw physiological changes and toxicity changes that we could reverse with anti-sense oligonucleotides in the dish.

So this is almost a patient in the dish that's now ISIS Pharmaceuticals -- very unfortunate name, ISIS -- anyway, that ISIS Pharmaceuticals have taken now, and are taking now, through to clinical trials based really on a dish model without even having a transgenic mouse line.

So we are getting to the point where we can do this in the dish. And finally, we also did one

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of the first molecules of Huntington's disease. It's a huge consortium, NIH-funded effort, which I had the dubious task of pulling together. We didn't want to give any authorship to anyone, so we called it -- the HD iPS Consortium was the author. But it had over 70 authors on it and seven different labs.

But we really showed nice, specific changes in Huntington's disease neurons that we could model in the dish. And now, again, NIH in partnership with a number of companies are using this model to screen drugs for Huntington's disease, which has been very difficult to model in mice. In fact, there were very few models in the mouse of neurodegenerative disease in humans that are really efficient. So this was another new model.

We're getting more sophisticated now with these models. We can put these cells into different chambers. And, for instance, we put cells in this one like muscle, and we can put cells in this one like DRGs and connect them together, and the spinal cord in this one with microfluidics.

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I think this opens up the opportunity for modeling human conditions such as pain. We can isolate DRGs. We can, perhaps, then combine them with spinal cord cultures and look for models of neuropathy or other interesting models.

Now, we have access to the cells, we can do it. And if there are genetic forms of neuropathy, we could actually have the patients' own cells in the dish and their DRGs connecting with the spinal cord.

We have recently taken this to the next level by collaborating now very strongly with the Wyss Institute set up by Don Ingber at Harvard with a $250 million gift from Wyss, who is a Swiss entrepreneur. What they are doing is actually making what they call an organ on a chip. And DARPA and I think FDA are funding a chunk of this work.

Again, really, the long-term goal is to create much more sophisticated organs. And as an example, this is kind of the size of a memory stick. They have a microfluidic system where they

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put endothelial cells on one side, in this case, lung cells on the other side of this membrane.

This microchip essentially is a mini-human lung. If you go in and blow this up, they're very, very clever engineering. This is the endothelial layer. This is the lung layer. They blow air in along the top and they put blood in along the bottom. And then these two things pull and stretch the tissues to simulate breathing.

In fact, the movement in tissue culture is very important. We haven't really appreciated this, but when you move the cells, they start behaving more like real lung endothelium. If you do this with gut tissue, they start creating microvilli and producing more like gut tissues, so movement and mechanical forces are important.

I think, with DARPA funding, they've now put five of these different organ systems together in one incubator, so we're trying to reconnect and mimic the human body through these kind of technologies. Now, this is very cutting edge. We're providing now stem cells to move into this

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model. And in partnership with these, we hope to create some interesting models on a chip of different organ systems.

I would really love to move this into pain as soon as we can and work with anybody here who would like to collaborate. For actually trying to make some sort of pain-on-a-chip models that we could think about how systems interact to create normal models of pain, I know the field needs some boosting, I think, in certain areas. And this may be an area where we can really work hard to get newer models of pain that will be more relevant to human disease. So that's my in vitro part.

The last part of the talk will be focusing more on the in vivo side and the experiences we've had with FDA moving stem cells to the clinic and trying to think about how these stem cells will be used.

I think, before I go any further, I'm just going to acknowledge Steven for the work he used to do in GDNF and still does perhaps. But these kind of papers on GDNF and pain were very important a

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few years ago in thinking about how we could use GDNF to treat pain.

I've been interested in GDNF for many years, and I'll show you why in a moment, because GDNF is part of a larger family of molecules, all of which interact essentially with the RET receptor. They float along in this little graph. They bind with the RET receptor. And this family of molecules has various effects in models of pain and, indeed, in many neurogenetic diseases.

Once the GFR alpha-2 receptor, neurturin, or GFR alpha-1 and GDNF are bound. This activates a whole set of intracellular signaling pathways, which essentially, I'm interested in cell survival and neurite outgrowth, but also, through this mechanism, interact with pain pathways in a significant way.

A gene is great, but it can't penetrate the blood-brain barrier very easily. And so it needs to be infused, many studies infused into the CSF. That does lead to Limette syndrome and other issues, non-specific issues that are related to

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GDNF. So actually getting it into the spinal cord has been very difficult.

Into the brain, as we showed some time ago, we can get that GDNF into the brain. And we, in this study, with my long-term friend, Steven Gill, put together a project with Amgen supplying the GDNF a few years ago, where we infused GDNF directly into the brain of patients with Parkinson's.

One of the first uses for GDNF, because it protects dopamine neurons, was going to be to treat Parkinson's disease. They struggled. They put it in the ventricles. They did not have a paper, but it caused all sorts of side effects and no real effect.

That's because to penetrate deep into the brain tissue, the GDNF really needs to be in the brain -- and I'll get back to this -- or the spinal cord because the human spinal cord is so big and the aqueduct is more or less not there. Getting GDNF to the motor neurons, I'll mention in a minute, is different. So we decided to be a little

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gung ho in England and did a small trial of five patients.

We did see a nice change in the Parkinson's scale in this paper. And we also saw some PET changes in the patients that we infused GDNF, where the dopamine levels seemed to go up where we were infusing the GDNF, so very encouraging. I could show you videos of miracle patients, but it was an open-label trial.

Parkinson's is notorious for false positives. It's actually a disease that dopamine neurons are a reward mechanism. So if a patient thinks they're getting something, they will jump out of bed in the morning and respond if they thought they were getting the drug.

So we were very cautious in our interpretation. This needs to be double blind. Amgen were less cautious and very excited and ended up doing a very large double blind trial in 40 patients, which failed. I'm not going to go into the details. Maybe in the discussion, this could be a discussion of maybe how not to take

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preliminary trials into the clinic further.

But they use a different catheter. The catheter they use was an open catheter. We used convection-enhanced delivery, and it's just a simple host pipe analogy. If you're trying to get a drug into the brain and you put it through a very wide-bore catheter, it's going to reflex back up. If you put it through a very tight catheter under pressure, it sprays out. You get convection-enhanced delivery, which is what we did in our initial trial.

So that trial failed. We still don't know if it could work. Steve Gill has just currently started a new trial in Bristol, in England, using the same catheter with a new set of patients and blinded. And so we'll wait and see what happens because we still don't know if GDNF works with Parkinson's.

There's always the cumbersome aspect of having to make the recombinant protein. It's very expensive. So I was always kind of keen on other delivery mechanisms. This is where my worlds met,

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the GDNF world and the stem cell world came fused together. And I thought, if you can make stem cells secrete growth factors such as GDNF, you get over the delivery problem. I think maybe the stem cells will do something useful on their own.

That really got me into the era of stem cell biology. I started with Parkinson's back in Cambridge with Anders Bjorklund and others, trying to get dopamine neurons for Parkinson's, but then rapidly developed into a different area.

Of course, I've mentioned pluripotent cells. We could use these for delivering cells into the brain. And maybe you'll hear in a little bit from Allan about tunnel suffix cells being used for this, for interacting with neural circuits.

But the idea usually is, you take these pluripotent cells, you make them into progenitors, and then you get your three types of the brain, the neuron, the oligodendrocyte, or the astrocyte, and use these for repairing damaged brain tissues.

Now, neurons are fine. The problem is, you need to really have integration into circuits. And

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again, you might hear more about this from Allan in a moment.

For ALS, which I'm going to talk about, you not only need an integration to a circuit, you need to get that axon of that motor neuron to grow about three feet out to the muscle. Now, this has been a huge problem for the field, for motor neuron replacement.

Again, this is just to reference Allan's work he'll probably talk about in a moment. There is some beautiful data on neurons being able to interact with pathways in the dorsal spinal cord to modulate pain.

So neurons are important. Oligodendrocytes is the other cell type we can make. Here, they're being used now in a trial in New York, Steve Goldman, for remyelination of multiple sclerosis, which is because myelination goes and the oligodendrocytes may remyelinate. And also for spinal cord injury, remyelination is being used by Geron, a company in California, that just reactivated a trial for these cells in spinal cord

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injury.

Here, they're actually trying to remyelinate areas of the cord, which are demyelinated in the injury, in hope to get a little bit of function back in these patients. They're not trying to restore the whole circuit, the motor neuron circuit in these spinal cord injury patients. They're just trying to get a bit of remyelination, which is a very interesting approach. It would be nice to get more patients into that trial.

Finally, the astrocyte. Now, this guy is the poor guy of the brain, but there are three more astrocytes for every neuron in the brain. When they looked at Einstein's brain, they chased it all around the world and finally analyzed it. Guess what? He had more astrocytes than any other human known to man, a whole load of astrocytes. Nobody knows why.

I can tell you, the astrocytes are probably the most important cell in the brain. They modulate neurotransmission and may well modulate pain. They have a whole role of functions. They

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interact with the blood-brain barrier. So I converted to astrocytes about 10 years ago.

I always tell my students, publish in Glia Journal now, not Neuron, because in 10 years, Glia will have a bigger impact factor than Neuron and it's easier to get it. But nobody takes any notice on me. We'll just have to wait and see if history shows that's true.

So the astrocytes are very important. And they may be sick in certain diseases. We always focus on the neuron, but in ALS, there's good evidence that the astrocytes are sick, and I'll get to that in a second.

They can also be used to deliver drugs. They are a very nice cell. They migrate in the CNS and the spinal cord. So if we engineer them to make drugs, maybe they can help increase plasticity, increase protection of neurons in the brain, and of course reduce pain if they are releasing GDNF. I don't have any data to show this can happen, but I do have a lot of pre-clinical and clinical data to suggest it's now possible.

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We haven't really moved so fast with these pluripotent cells because I think, as Spiderman once said, "With great power comes great responsibility." So, yes, these are very powerful cells, and that means we have to be incredibly careful about moving to clinical trials because one tumor in one patient would set the alarm bells off with the FDA correctly.

So we have to be very cautious. And people are moving these cells into the clinic, but it's a lot slower than another source, which is fetal brain tissue, which has been around for 20, 30 years. People have been growing stem cells and neural stem cells. And that's really the main amount of clinical data at the moment, is from human fetal tissue that's been driven down this route. Human fetal tissue is not as plastic, but it is much safer to go into patients.

This is ALS. For those of you that don't know this disorder, the worst diagnosis you can get, neurologists can give you, probably is ALS, Lou Gehrig's disease. Of course, the baseball

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player had it and his batting average actually went down five years or two years before he actually had onset of symptoms, so there are things going on in your body and you're losing motor neurons. You compensate. And then finally, you go off the edge and get this horrific disease.

It's actually a complex disease. The motor neuron system starts up here. When I want to move my hand, I have to activate upper motor neurons. These upper motor neurons go all the way down to the cord. In a human, they actually go all the way down, about four foot, all the way down to the spinal cord, where they activate the second motor neuron, which is called the lower motor neuron. That's the one that goes out to the muscle, and that's what enables you to move, so really two circuits from your brain to your muscle.

In ALS, both of these neurons die. We don't really understand so much about the upper. I have a whole story on that, but I don't have time to tell you. But we do understand quite a bit about the lower. And in this lower motor neuron in ALS

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patients, you lose about 90 percent of these lower motor neurons. And probably when you get to 70 percent loss, you start getting paralysis.

So from onset to death, total paralysis, you don't actually die from ALS. You die because you decide not to go on a respirator. The total time is usually three years. That's the average. You start with muscle fasciculations. It moves from one arm to the next arm. And then about a month or two later, it starts in your legs maybe.

Everything becomes paralyzed until, finally, your lungs paralyze and you have to choose or your family chooses whether to go on a respirator. Usually, at that stage, people decide not to in the U.S.A., about 70 percent of people. Interestingly, in Japan, 70 percent choose to go on the respirator. Whether that's to do with healthcare, family, we don't understand the ethics behind that.

We don't know what causes ALS. It is completely unknown. There are no drugs. There's one drug called riluzole, which does very little. There's no drugs for ALS, death normally within

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three years. It'll happen between 30 and 70 years old. A majority of cases are sporadic, but like every other disease, neurological disease, there's a genetic form like Parkinson's, like Alzheimer's, all these diseases.

About 10 percent are familial and about 90 percent are sporadic. But the familial types, this SOD1 gene, if you've over-expressed a mutation in SOD1, you get a beautiful ALS model in the wrap. And this has been the real workhorse, where motor neurons die. And I'll show you that in a second.

There's been varied new genes and one called C9ORF, which are coming up in ALS, which may now account for about 50 percent of familial forms now that turn out to be associated with a C9ORF mutation, which is also responsible for frontal-temple dementia. So there's an interesting link here between Alzheimer's and ALS that's being actively investigated right now.

We haven't even got a name for this gene yet, it's so new. I mentioned earlier, we've modeled now ALS with iPS cells and have shown we

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can reverse some of the pathology caused by that gene, so that's a lot of excitement. The SOD1 gene is still the workhorse.

The pain pathways in ALS were of interest to me as well with regard to GDNF, having come from out of the Parkinson's field. And you've heard a lot of this yesterday; maybe yesterday, you've heard some of this.

But GDNF acts on many different levels in the spinal cord. Of course, the most interesting one here is the GFR alpha-1 sensory neuron pathway that can be modulated to change pain thresholds and sensitivity, a number of papers by Steve and colleagues, who have shown the effects of GDNF in pain modulation.

This is, again, the intact spinal cord with the motor neuron coming out to the muscle. This is from an old review we did. And really, in ALS, we don't know the mechanism, but, clearly, you can have the mutation, this SOD1 mutation. The astrocytes change. They become actually toxic and aggressive.

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The motor neurons retract from the muscle and the astrocytes also retract from the blood-brain barrier. So the idea here is to replace the astrocytes, where they can actually then restore function, because we're assuming that some of the cause of ALS is the death or damage to the astrocytes.

So again, we're putting the cells into the patients to try and modulate motor neuron function, and protect the neurons that are still there in the patients, because we can't replace the whole circuit. But also, we're going to arm them with GDNF, so the idea is, GDNF was shown by Chris Henderson many years ago now to be more active on motor neurons and survival pathways than dopamine neurons. So it's a well-known survival pathway for motor neurons undergoing degeneration. So this is the concept, genetically modify cells to release GDNF.

Which cells do we use? I won't go into a lot of detail, but we've been using cortical cells from fetal tissue now since the early '90s,

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actually, in Cambridge. And these cells can be expanded in EGF and FGF. We call them neurospheres.

We developed this novel chopping method. Martin Raff once told me that any cell, a single cell and culture, from a multicellular organism is abnormal. You should never clone a cell because cells in multicellular organisms are together.

So I took this to heart 20 years ago after dinner with Martin. Martin will convince you to do anything, those of you that know him. And we developed a new way. I thought, well, how can we grow cells without passaging them in single cells? And in fact, it's quite simple.

What you do with these aggregates that grow spheres is, you take a knife and you chop the sphere into quarters. If you do that, the cells within that chunk -- and this is about half a million cells, 500,000 cells in this aggregate -- the cells in these little trunks don't know they've been dissociated. In fact, they haven't been dissociated because you've chopped it.

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So this chopping method, we've pushed through now, and I'll show you, all the way to a GMP facility at City of Hope. And it allows you to grow these cells very consistently without dissociation over long periods of time.

We found, using this model, interesting things with these fetal-derived cells, is that they're not really stem cells. They're progenitors. And what we do when we expand them over a period of weeks and month -- and in fact, up to 70 doublings -- is that they grow, but they are changing over time.

First, they make neurons. If you take them, and expand them, and then you have to differentiate them on a laminate substrate to make them into neurons. If you grow them for around 2 to 4 population doublings, they make beautiful large projection neurons. If you then grow them for another 4 to 20, they make interneurons when you differentiate them.

If you push them past 20 passages, they make nearly exclusively astrocytes, particularly when

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you plant them into the brain. You can still make them into neurons in the dish, but at this later phase, they naturally change their ability to make different types of cells. And it's this that we capitalized on when we took these cells forward.

There are many companies that use similar cells, but they're not the same. Neural stem is actually in the clinical trial, I'll mention in a moment, Stem Cells, Inc., and Q Therapeutics. And they just use different variations on a theme, but none of them use a chopping method. And in the clinical trials they're doing now, none of them use engineered cells.

So these late passage cells are important. And now we've managed to actually grow these cells in a GMP facility and under GMP conditions. And we have a paper in JoVE that just came out if you want to see how you do this

With Patrick Aebischer many years ago, we started engineering these cells to secrete growth factors. And what we simply do is add lentivirus, which permanently engineers the cells, so this is

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integrating to release GDNF. And we've now shown a number, probably over 20 papers that we can grow these cells, make them produce GDNF. And they release GDNF under the PGK promoter consistently over long periods of time following transplantation.

So what do we want to do? In ALS, we are currently attacking here the lower motor neuron. We have a lot of data on the cervical. We can put these cells in the cervical part of the central nervous system, of the central nervous system spinal cord. We also are looking in the muscle to try and protect the muscle. And we're also looking at the upper motor neuron circuit.

So we have a very comprehensive lab focusing on all of these areas. And I only have time to show you some examples from the work that we do now. And I'll focus down here, really, in the lumbar spinal cord. A lot of this started with a graduate student in my lab many years ago, Sandy Klein. And we really worked until today, and still today, the rat model of ALS. It's a bigger model.

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We can do spinal cord transplants effectively in that model. And the neurosurgeons like it.

In this model, we get up to 100 days. Nothing happens. And then around 120 days, these animals start getting paralysis. The motor neurons die. Here are the motor neurons alive and they die over time.

So by 150 days, the rats are completely paralyzed and they over-express as a mutant form of SOD1. So this is a fantastic model of a neurological disease. I think it's one of the best models of a neurological disease that we have in the motor neurons die.

The bad thing of this disease is it only reflects about 3 percent of all ALS patients because the other 97 percent don't have a mutation in SOD1. So this is our dilemma with the FDA and with others. And I'll get to the data in a moment. Yes. It's an interesting model, but, no, it's not sporadic ALS. So we don't have a model of sporadic ALS, which has been a problem for us.

So what we do is -- this is how the rat

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looks at the end. I won't show you the video. They have come totally paralyzed. And we [indiscernible] requests, and we like to sacrifice them when they can't write themselves after 30 seconds. So that's our defined endpoint for this animal model.

Microsurgery, we put a microsyringe into the lumbar spinal cord. And actually, Guido Nikkhah developed this technique in Lund, when I was there with Anders Bjorklund, very elegant technique with a micro-syringe because we don't want to damage the cord. We squirt the cells in a little bit under pressure as well and they spread out nicely, and they integrate. And so I'll just show you we can follow them, actually, with MRI if we SPIO-label them.

These are five sites in the spinal cord just three days after grafting. You can see one, two, three, four, five sites here, one missed here. We only had four sites with the MRI scan. So we can MRI-track the transplants.

The labeling is pretty good. In fact, Pablo

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Avalos is a neurosurgeon that we collaborate with. He's training in my lab. He's fantastic, micron eyeball for getting these cells in the right place. It's not trivial, spinal cord surgery. So we do reasonably good. We do miss the target in the rat occasionally, but he's got pretty good at targeting.

This is an old-ish paper now, but it really shows you the concept. When we put one deposit of cells in the spinal cord, they can migrate up to 5 millimeters. These are cortical-derived neuroprogenitors in a human. They migrate. They like to migrate in the gray matter and the white matter, but they stop eventually. Here, they stop and about down here. But they get enough migration so they can fill an area up.

Every red dot here is a human cell. These are rats with human cells, so we can use human-specific markers. We have to use suppression and we actually use cyclosporine. But the key thing to see here is in this animal -- and they are releasing GDNF. So this is human nucleus staining

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for the human cells. And you can see they stay on one side of the cord. This is GDNF staining. You can see there, where the cells are, they're releasing GDNF, only where the cells are.

Here is the survival effect. This is the same animal. One side doesn't have a transplant. I think you can see all the motor neurons are pretty much gone. And here's the transplant side, and you can see the motor neurons are still alive.

So we have reproduced the survival effect of GDNF release, the astrocytes are made, and we get increased survival.

I moved to L.A. I was very worried it wouldn't work when we repeated this in Los Angeles versus Wisconsin, and it works in L.A. Here's the same effect, a good significant increase on the ipsilateral side, where the GDNF cells are on the number of motor neurons, shown up here.

Really beautiful transplants. They kind of crawl in around. They love the degenerating environment. The stem cells differentiate into nested positive and some GFAP-positive cell and

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wrap themselves around the dying motor neurons. These red colors here are the human cells in and around the dying rat motor neurons, and again, releasing GDNF along the way.

So this was all great. We were headed for Nature Medicine, and we got beautiful cell survival. The problem was, in these animals, we couldn't get functional recovery. So even though the cells were surviving, long story short, the axons were still withdrawing from the muscle. So we got increased survival, but when we did behavioral assays, the rat still became paralyzed at about the same time as a non-transplanted animal.

So it was very difficult to get a high profile paper because you want the whole story, but that was what it was. And we actually showed that number of innovations were less in the ALS model, and the GDNF level of the spinal cord couldn't correct that.

In other studies I don't have time to tell you and publish now, we've actually put GDNF in the

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muscle at the same time, and we can restore function with GDNF in the muscle at the same time. However, we don't know why we can't get full recovery with astrocytes just in the spinal cord. But as I mentioned, this is not a model of ALS, sporadic ALS, and that's our patient target population.

Also, the timing is difficult in xenografts. The human cells take a long time to mature, and the rat goes down with ALS. We have about 15 days from onset to death. So we have a mismatch here. This is a real problem.

Should we go forward? I think yes. The cell protection is very reliable, very robust. Yes, they don't connect to the muscle still, but again, in ALS, in patients that don't have the mutation, we have no idea of the pathology of the mechanism. And so it could well be that, in ALS, this technique would also restore function and keep the cells innervated to the muscle. We'll only know if we try it in patients.

So this led us to our CIRM. And this is the

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California agency, no accident that I moved to California. My wife is originally from Los Angeles. We've been in the frozen tundra of Wisconsin for 10 years, so that was certainly one reason. The other was Cedars-Sinai and how fantastic it is, but the third one is CIRM. They have $3 billion for stem cell research, and it has to go more or less to translational projects. And there is nowhere else in the world that we could get a $17.8 million grant to move from concept through to patients unless we had a CIRM grant. We have actually expanded these studies, even since writing this grant, that we got two years ago.

So we want to expand cells and then replace astrocytes. The work plan is now -- and I'll update you a little bit. We've been making these cells now that I've showed you at City of Hope, clinical grade. We use the National Gene Bacteria Labs to make virus, and we are now in the process. It was two years. It's now one year away from filing with the FDA, an IND with the FDA, to do this trial in human patients.

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I'll mention the trial design, but it's essentially phase 1/2A. I'll show you how we got around the blinding issue, by doing unilateral transplants. We're collaborating with NGH for the data monitoring. And there will be three sites initially, Cedars-Sinai, Emory, and California Pacific Medical Center, which are big ALS centers.

For those of you who are in clinical trials and design, this is the normal procedure. First, we have to make the cells, clinical-grade cells. Then we need to do the dose ranging in animals by distribution and toxicology studies, which are currently underway. We need to do pig as large animal, shows safety as well for this product, and then follow the IND.

Master-cell bank is now in the final phases of production. We did a research lot at Cedars and we did some of our pre-clinical dose-ranging studies with those cells releasing GDNF. And now we've done a process-comparable lot, and we're in the middle of the final run of a GMP grade of cells secreting GDNF, human, that will be compliant with

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FDA regulations for going into humans.

We are willing to share the drug master files with other groups, so if we were collaborating with a pain group, we'd be very happy to share. We're not commercial. I kept away from patents on this technology, so in order that we can actually share and collaborate with other groups; although some aspects of GDNF and stem cells are under different patents. But my feeling is that let's get something to work academically, and then license the technology out to whoever feels they have the best patents in the areas we're working in.

So we continue to move ahead academically. As I'll mention, we're not even scared to change the cell source from fetal to iPS, if that was the best way forward, again, avoiding the patent issues for now.

I'll just mention, this is how we do the cell production. We end up with about 1,000 vials of product, which is simply thaw on the day, rinse, and inject into the patient. We've shown in all

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pre-clinical studies, we can get that product of GDNF-secreting cells that survive for and inject into patients.

This is, again, the series of things that we have been doing. And I won't run through them all, but we can maybe, in the discussion, see sort of the process of going from an idea into the clinic. Just to give you some updates, we do get a nice dose response in a number of cells. We've done different doses ranging from 20,000 to 2 million cells in a spinal cord. That's our maximum feasible dose, nice correlation with cell survival.

We also get a nice correlation with GDNF release. Remember, we are putting more cells in, so we're getting more GDNF. We do ELISAs on the spinal cord. And we see that the amount of GDNF correlates with a cell number quite nicely. The maximum feasible dose here, we get up to high levels of GDNF production in the spinal cord and the vehicle. Basically, these cells make no GDNF on their own. They have to be engineered, and then

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they start secreting it. So they don't produce endogenous GDNF.

Again, just from the different doses, nice and consistent graphs, getting obviously to the endpoint. When we put in the quarter-million cells per microliter, it's almost a solid transplant at the core, and the cells don't migrate as much. Lower doses, we actually get more migration. So we're actually coming down to a dose between 10,000 and 50,000 cells as being the optimal dose.

We see the same with the dose response, rather flat curve. We were surprised. Even a low dose has a threefold increase in the number of motor neurons surviving in the spinal cord. So even a little bit of GDNF secreted from these cells is effective. If you put the cells alone, you get no effect. We have done this many times. So they have to be secreting GDNF in order to have this effect, and we think it's synergistic with the cells themselves.

In behavioral aspects of the work -- and I wanted to get to the pain aspect -- we don't see

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any major changes of these doses in the GDNF, but we did do the pain thresholds and sensitivity. Given the effects of GDNF on pain, we wanted to check that it's not having adverse effects on pain.

We did the usual. You guys are very familiar with this, much more than I am, von Frey, the Randall-Selitto test, the flinch-jump, just to see if the GDNF-secreting cells increase pain in these animals. And we're in the process of analyzing all this data, but I will show you that we didn't see any overt changes. They're actually at time zero. We didn't train these animals in the von Frey for weeks before. We only did three tests. We found there was a continual training effect, where they wanted to withdraw their paw over time, but then it stabilized.

The bottom line is, with the von Frey, there was no significant differences between any of the groups. So we didn't suddenly see a spike in pain in the maximum feasible dose, which was releasing a whole lot of GDNF.

We didn't look at the opposite, either. We

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could have been having a pain modulation of reducing pain, but we haven't used these cells in any models of pain. We would love to if anybody is interested in collaborating, to see if we'd get a reduction. And we're targeting the ventral horn, not the dorsal, with this study, although some cells do spread out because you can see into the dorsal area, which is why we wanted to assess pain.

With the paw pinch, the same, no physical differences between the groups. There's a lot of data in here again. Just the bottom line is, we didn't get a significant difference or increase in pain using this assay; and finally, the finished jump, again, no difference in pain.

So we don't think that these cells are overtly increasing pain. We haven't tested yet whether they may decrease pain if we used a pain model. So there are my pain slides. I got them in. I'm almost done.

So basically, the other issue that we've had is how to get the cells into the spinal cord. I think this has been holding the field back a lot.

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Now, Nick Boulis and I developed a frame many years ago, which is a bit like a Meccano set, but we use this frame for pigs to show that we can target the spinal cord nicely here at GDNF-secreted cells, the pig spinal cord injected using the stereotaxic device, which you basically bolt onto the pig or the patient.

It's actually a bit like a Meccano set, so if you're a neurosurgeon, you love this kind of stuff. But we wanted to make a simpler device for surgeries and the cord for either spinal cord injury, pain-related rhizotomies, et cetera.

We are in the process of developing a minimally invasive system that has an X-Y arm. We've done now about 20 pig surgeries using this device, and it looks to be very successful. And we will probably be using this device in the clinical trial next year, but we're still in the process of finishing up the ISA forms and the requirements of using this device in a clinical trial, so it's an interesting device.

Finally, the trial itself -- and this is the

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last point to make -- is an 18-patient trial, but it's unique in that the nice thing with stem cells releasing a growth factor is, rather than a pill, which you have to give to the whole patient, we can localize the delivery. And this is really unique, and it's allowing us to do a very interesting trial design, and that is that we're going to do a unilateral transplant. So the neurosurgeon will put the cells either into the right or the left side of the lumbar spinal cord.

Obviously, in ALS, it's interesting. Even though the rates are very variable between patients, if you get ALS in one leg -- and what I mean by that is in some patients, it may take two years, and in others, it may take 10 years to become paralyzed in a leg.

The neurologist will tell you, though, if you're a fast progressor and it goes fast in one leg, it will go fast in the other leg. And if you're a slow progressor, it will go slow in both legs as well. So in a way, either leg is a control.

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So the idea of this design, if the IRB approves it, is we do a unilateral injection, which would only affect one leg. The neurosurgeon will decide, based on blood vessels, which side to go in. The neurologist will be blinded. The patient will be blinded. So in effect, the patient and the neurologist won't know which leg had the transplant. And the beauty is, with paired statistics, we can get a lot of power because we'll be analyzing the amount of the rapid decline in paralysis over time in one leg versus the other.

Using paired statistics, we can actually get a lot of power in the analysis. In fact, in 18 patients, we can actually get significant differences in onset. And if we can get a significance in this small trial, it'll give us a lot of momentum to move forward to doing cervical injections and being more ambitious. So that's our goal.

There are other trials going on in this area, but really only two main ones. One is Mazzini in Italy. She's been putting mesenchymal

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cells into the spinal cord of patients. And we're not quite sure of the mechanism here. They're not engineered to release GDNF. They may release some growth factors. In our hands, these cells don't survive. If you put mesenchymal cells in the spinal cord, they die within about two weeks. We have no idea. There's really not a lot of pre-clinical data to justify this, but they have moved ahead in Italy.

There's a neural stem company that's based on a lot of work we did early on with Nick Boulis. They've done about 30 patients with ALS now. They're in a phase 2 currently in Michigan. So they have shown that you can safely put cells in the spinal cord, which is very good for everybody. We have our trial at Cedars, which hopefully we will get off the ground next year. And then there's Q Therapeutics and Angelo Vescovi in Italy has done a few patients.

So we are able to, remarkably in humans, transplant cells into the spinal cord and not make things worse. These patients, none of them, got

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worse. There are some claims that some of them are getting better. We're still waiting to see the clinical outcomes of these trials. But I was so impressed that you can actually do this. I was always worried initially, if you put these cells in, they're going to interrupt spinal cord function. You're going to get pain. You're going to get all sorts of symptoms; nothing in any of the patients. So it's quite remarkable, I think, these studies.

They are also moving onto cervical. They were so happy with the results, FDA gave them permission to move to cervical. They have now done a number of patients in the cervical areas to help with breathing. And they'll probably be moving bulbar at some point.

We're out here. We just got our funding from the DoD to inject GDNF into the muscle. That's kind of interesting as well and a whole other story. We're using AAV here, gene therapy approach, not stem cells, to try and restore this connection that I mentioned was missing. And

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finally, we were working up here. You could restore the whole function here. If the upper motor neuron dies, you're still paralyzed. So we have some ideas up here that I can discuss at the break.

This platform product, I just want to emphasize, we're also looking at for Huntington's disease, we're looking at for retinitis pigmentosa, and macular degeneration. These cells work very well in that context, and we have a grant from NIH now to put them in the back of the eye. They seem to slow down degeneration in that disease.

In stroke with Gary Steinberg, and I've got a joint appointment up there at Stanford, we've shown these cells can integrate after stroke, and increase plasticity, and survive. And the astrocytes are doing something good, not secreting GDNF in his case, just normal wild-type cortical derived progenitors.

Where I started was in Parkinson's, really was keen to use these cells to deliver GDNF in Parkinson's. Why didn't we move ahead? My feeling

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is that the one thing with this technology is, we can't regulate the GDNF release. If you put the cells in, they're going to make GDNF for the rest of that patient's life. And they survive and they release.

So I didn't want to have a situation in four years with Parkinson's, where the patients are getting side effects and we couldn't switch off the GDNF. And that was rather similar to the fetal transplants, so you know that field. It worked very well for Parkinson's. Twenty-five percent of patients got dyskinesia, is they couldn't switch off, and they had to do pallidotomies.

To get over this is possible and for pain as well. And the way to do it -- and we've been trying five years to get it to work. We think we're close now. It's using inducible system. And the old doxycycline systems weren't that good, and we tried them. They work in vitro but not in vivo. We're now coming up with newer ones with Josh Breunig, a graduate student. We now can regulate GDNF release very nicely, no doxycycline and

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doxycycline.

So if you get doxycycline, you switch on the secretion of GDNF from the cells. And that, I'd be happy with in Parkinson's. And then a Parkinson's patient takes a pill to turn on the GDNF release, and then turn it off again if they don't want the GDNF anymore, if you find that it's only useful for a while or need it for a while.

We've got a cool study. We've actually done this in vivo now, transplanted cells. And the animals that received dox are lit up here with luciferase. We attached a luciferin gene, and the animals that didn't get docs off. So we can actually, in vivo now, switch on and off the GDNF secretion.

So this is kind of cool. We need to move it into primate studies and are currently looking at doing that with this construct. And we'll probably use this construct for ALS eventually as well. ALS, I'm not so concerned about because you're faced with dying after three years. I'd rather have a side effect in GDNF than not be around

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anymore. But ultimately, we'd love to regulate it in ALS as well, so I think we want to take this forward.

I think for pain, it would be kind of interesting that we can actually now modulate pain; if we can put these cells in, that we can actually adjust the GDNF levels. We can put them in, and turn them on and off at will, and see how that affects pain and pain thresholds in animals. And I think that would follow on nicely from Steve's work and others with GDNF in the future.

So this is all fetal tissue, just to say that we have this new technique now, where we can, from iPS cells, get these. We call them EZ spheres, and in England, that doesn't mean anything, just E-Zed [ph] spheres. Anyway, good old USA, that's EZ spheres. It's a bit like soccer. There are always problems. America seems to be doing quite well for soccer at the moment, Britain not so well.

So we make EZ spheres, and those spheres can then be used for all the things I talked about.

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And the interesting thing is, first of all, we can make this a GMP process. Again, we use chopping to make these EZ spheres. We lift them off the iPS, and EGF, and FGF. But more importantly, we have generated now, just published a way of going from an EZ sphere from an iPS culture to something that's very, very similar to a neural progenitor cell that I've talked about from fetal tissue.

We worked very hard to find the conditions where we can generate the progenitor that's almost identical to fetal cells, but now it comes from iPS.

So now, we don't need fetal tissue anymore theoretically. We'd like to try the trial with fetal tissue, but then behind that, we have a wave coming where we'd like to try a trial with iPS-derived cells because that's ethically easier, and we have more control over the manufacturing of cells from iPS. So it's a very exciting time in the field.

These cells, by the way, do -- again, another little paper we just published. The

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iPS-derived fetal progenitors look identical to the fetal progenitors, so the iPS cells made into fetal progenitors look very similar. These are fetal-derived progenitors that I showed you the pictures of. And these are now progenitors derived from iPS cells, induced pluripotent stem cells, and they look almost identical, the way we've manufactured them, to the fetal-derived cells. So we're getting away from the need for fetal tissue as well.

This actually allows for autologous transplants. And I'm off to a meeting in a couple of weeks to talk about whether we need that for the brain. But for the PNS transplants, you could use the same patient cells. I think you wouldn't have to use suppression, we hope. There's a whole story on that as well, but that would maybe help. We don't need to use fetal tissue or destroy embryos.

The downside is, in the brain, neural progenitors may not need rejection, may not reject. There's a blood-brain barrier protection from rejection. So do we even need to go to the effort of autologous for CNS transplants? That's a big

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topic of discussion.

If you develop them from an ALS patient, autologously, maybe the after-science already, they're going to have the disease. It doesn't look like it. It looks like, when we reprogram, we wipe the slate clean, and the disease may come back in 40 years. But if you get another 40 years of a new astrocyte, that's probably enough.

The regulatory pathway for autologous is very complex, but if it works, we'll work out the pathway. If we get something to work, we don't need suppression. Remember the cost of suppression in these trials is huge. You have to weigh that against the cost of manufacturing an autologous way.

I'm not worried about autologous manufacture of the cells if they work better than non-autologous cells. But we will have to do individual GDNF infections, which will be a pain. Sorry for the pun. It would be difficult, but we could do it. It would be expensive, but again, if it works, we'd do it.

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So I think the first part of it would be allogeneic iPS-derived cells. So use iPS, but make an allogeneic line that can be used for many, many different uses. And that's what we'll probably be able to have and distribute.

The last couple of slide -- I know I'm running a little long. But I just thought I'd finish with kind of where we are with this iPS technology and try to tie those two, clinical side together with the research side.

This is the old Waddington slide of totipotent cells. Up here, for embryonic stem cells and now induced pluripotent stem cells that we can make, they go down into these pathways, which essentially used to be differentiated tissues. This may be skin. This may be blood. It may be bone. So you have this sort of differentiation. And it used to be thought this was the end and you couldn't go anywhere, but now we find you can go back.

I thought this was a bit boring, so we updated this a few years ago. And this is my

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pinball wizard. Who remembers the Who? I'm sure Steven does. And Shinya Yamanaka is kind of our pinball wizard for iPS cells.

Anyway, my contribution here, Dhruv Sareen and I did this. It was really the sperm and the egg, sort of British humor. Anyway, this is the fertilization part. And now, the pinball goes up. Up here, you're pluripotent. But when you start going down and falling down -- and essentially, embryonic stem cells and iPS cells have an energy. You've got to keep them pluripotent, which is similar to the pinball machines. They've got a lot of energy, inertia up there.

Then it differentiates, and differentiation is going to take off the energy. And they start to spontaneously differentiate. But you direct them with growth factors, and with transcription factors, and here, small flippers, down into the different lineages, endoderm, mesoderm, ectoderm. And eventually, they get more and more differentiated until you flipped them with the Oct-4 flipper, and they become pluripotent again.

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So that's kind of the idea. And it's actually not a bad analogy. Sox-2 is another pluripotent factor. Even within tissues, like the pancreas, you can have flippers that are smaller transcription factors that drive from a secretory cell to a non-secretory cell. And in fact, what people have found now is, you can take a skin cell, for instance, and convert it directly into a neuron by using two sets of transcription factors. You don't have to go back to a pluripotent state up here.

So it's a very exciting time. And I think it really tells you about DNA. Most of our DNA is the same. I'll take out blood cells from that -- or cancers. Obviously, terminal things happen to the DNA. But healthy cells, most of the DNA is probably the same in every cell in your body. So if you can manipulate it with iPS technology, you can go back to a pluripotent state.

So to conclude, I hope I've given you a lot of information, but modeling with iPS may be the biggest thing. If we can model pain in the dish

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with human cells, it's going to be very, very powerful to test drugs because stem cells can secrete factors that help motor neurons. If you combine that technology of stem cells with growth factors, those are combined stem cell gene therapy. It's like putting the troops in, but giving them the weapon, which is GDNF, so they can actually help modify the area that they go into.

Localized delivery of cells is important for pain, I think, as well. With stem cells, you can target areas very specifically. You can do that with genes as well, you'll probably hear in a moment.

The nice thing with stem cells is you don't have to modify the host cells as you do with gene therapy. You put in a new cell, which has its own complications. But at least the host cells remain the same, and you just engineer the cells that you put in. And there's pros and cons with both of those we can discuss at the panel.

So the localization, I like. Autologous is possible now with iPS cells. I still think that

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slow and steady win the day. Rushing into trials is not the right way to go, and I really appreciate the interactions we've had with FDA. In fact, I was in this room, I think, last year with a bunch of ALS patients trying to lobby the FDA and to going easy on us, to get trials going.

I think the consensus is FDA is doing a pretty good job of regulating this field because, when you mention stem cells, it's like snake oil. And a lot of companies want to jump in and charge you $40,000 for their stem cells, and that's not good because a lot of those therapies are not proven.

So I think we're on quite a good balance. Obviously, we'd like a little more flexibility in ALS, in a disease where there is nothing with the FDA to try and help us move trials forward. And I hope that some outcomes from the discussions we've had with them will allow this to happen.

So most importantly, this is the team that worked with me. I get the fun job of giving these talks. But really, we've had a lot of

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collaborators over the years. I'd particularly like to thank Jamie Thomson for the original, getting into iPS cells when I was in Wisconsin. The cells that we make are really true -- Sareen. And this huge group now is working on the ALS project led by Gen Gowing and Brandon Shelly, who actually just got married, which is rather nice. The lab, sometimes it works and sometimes doesn't. So they kind of run the lab together.

So I'll stop there, and I'll thank also our funders, of course, which is CIRM, the NIH, and Cedars, and the ALS association. Thanks very much.

(Applause.)

DR. PORRECA: Thank you for the fantastic job. Questions?

DR. MCMAHON: The ALS studies in the SOD1 mice, is delivery of GNF from the muscle sufficient to protect motor neurons as well as maintain terminals?

DR. SVENDSEN: Yes.

DR. MCMAHON: But if not, why not? Is it something that you have to overcome additionally in

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the cord?

DR. SVENDSEN: So in the model that we use, we get -- in all these models, eventually, the rats succumb to the disease. But GDNF can delay that by about 20 days if you deliver it to the muscle with a virus or with actually a cell delivery to the muscle. So you can delay it about 20 days, but then they finally succumb, even though they get a lot of GDNF in the muscle. But early on, yes, the GDNF is retrogradely taken back to the spinal cord, and you do get increased protection in the spinal cord itself.

We're currently doing Jewell transplants, which is cells secreting GDNF in the spinal cord along with cells and GDNF in the muscle. And we're in the middle of those studies, and we're really hoping that that will extend it maybe 100 days. We'd love to get to the point where we keep the animals alive, but I think those combined therapies, muscle and spinal cord, is going to be the eventual way of getting a bigger effect.

But let's just go back. In ALS, again, this

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SOD1 model is not sporadic ALS, so we're not sure of the mechanism in sporadic ALS. Yes.

DR. MCMAHON: [Inaudible – off mic] -- the increasing toxicity of the astrocytes in the animal?

DR. SVENDSEN: I think it's a very, very aggressive model. To get the rats to actually show the degeneration, they had to put 12 copies of the CD1 mutant gene in. And the brain is full of mutant SOD1. So I think the model is so aggressive --

DR. MCMAHON: Everything's dying.

DR. SVENDSEN: Everything is dying. So you can get protection, but it's very limited. But I think, if we do Jewell transplants, if we could get it to 50 days, that would be a first for the field because most people manage to get these models out maybe 20 days.

DR. MCMAHON: But it will be very attractive to have a therapy that just involved an intramuscular injection.

DR. SVENDSEN: Much better, much easier.

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And in fact, that trial may get into the clinic before the spinal cord one if we run into any difficulties with the spinal cord trial. Yes.

DR. BASBAUM: Thank you. I thoroughly enjoyed it. One simple question, and then one a little more complex. Insulin growth factor, there was obviously that large trial, and it was controversial. But I was kind of surprised that you -- have you ever tried this with insulin growth factor, was the first question, which I thought was the one that was going to save the motor neurons.

The other question is, you're putting GDNF in the cord. Is it producing or have you looked to see if it's producing any sprouting from, say, afferents or something else that might be RET positive?

DR. SVENDSEN: So the first question, IGF1, of course, we followed Feldman's data in the IGF trial, which failed, unfortunately, in the skin. IGF-secreting cells, we have made them. They don't have an effect in this model, in the way that GDNF does. In fact, we've got a paper coming out, back-

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to-back comparisons of VEGF, IGF1, and GDNF. And the GDNF wins out on cell survival.

The only one we're interested in is CNTF. In the old days, CNTF was another molecule that Aebischer and others promoted. And that seems to have an effect at the terminal and the -- from the mouse data, from the motor neuron, and at the terminal.

So CNTF may sprout, and we're in the middle of a CNTF-secreting line put into the spinal cord. But there's nothing dramatic happening even with CNTF as far as we can see. So I think the answer is, put in the growth factor in the muscle as well.

DR. MCMAHON: [Inaudible – off mic] -- well, because of the cachexia.

DR. SVENDSEN: Well, the toxicity of CNTF -- see, all of these drugs were originally put into the CSF. And in ALS, many years ago, they tried them all. And they had side effects of toxicity, but they never actually did anything in ALS. But now, we're actually putting the drugs around the motor neurons, where it's needed. We'll

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see the actual effects, I think, without the side effects, because we don't get secretion outside of the area where you want it, which is where the cells are.

So I think the side effects, to a large extent, may disappear, unless they're relevant, of course, to the motor neuron and causing sprouting. Now, we didn't see aberrant sprouting. We looked very carefully in the dorsal horn to look for any GRP-positive fibers. We didn't see anything massive, and I think it's because that's an intact system.

So maybe, in an intact system, if you put GDNF in, you won't see anything. But if we did a crush or we did something out peripherally, then I think the GDNF would interact, and maybe Steve can talk to that as well later in the discussion about that it's doing there. But we didn't see any overt sprouting. Most of the GDNF was in the ventral horn, though, not so much in the dorsal. Yes.

DR. GLORIOSO: That was a lovely talk. What is the viability of the cells that you implant, and

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what's their immunogenicity?

DR. SVENDSEN: So the viability is around 20 to 30 percent of cells that we put in. So if we put 50,000 cells in, after 10 days, we'll see around 10,000 to 20,000 cells absolute. Then they actually divide. Over the first six weeks, we get maybe one or two divisions. And then, if you look at 12 weeks and after 50, the division completely stops.

So they go in as a progenitor, and they actually divide a couple of times. So the total number of cells is almost the same as you put in. But there's immediate shock and death. And then they divide a couple of times, and then they stabilize.

Are they immune? Well, they're immunogenic. This is xenografted. If you put them in with no suppression, they will completely reject. The cyclosporine works, and it actually works very efficiently in the rat model. We've had huge problems in pig suppression, which is a whole other talk. We have had to use very, very severe

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suppression regimes in the pig, try suppression to get the cells to survive, and now, we've got it. But the rats seemed fine with not oral cyclosporine, but IP cyclosporine daily, 50 mgs per kg. That will keep the cells alive.

That's a whole other topic discussion. We are planning to do pretty severe immunotherapies, just like we do for organ transplants, for the first set of patients, assuming they're going to have a rejection issue.

But again, the field is very -- there's a whole meeting at SOMA, actually, in a couple of weeks, discussing what the best approach for immune suppression is. Patients don't like the very strong suppression, the ALS patients, but that's the way we're going to try first of all, I think, because we don't know in an allograph what would happen. There's no model of allogeneic human transplants, and we can't use mouse.

Yes?

DR. FETELL: This is an exciting talk. I was wondering, it seemed like, in your early

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experiments, the animals weren't recovering behaviorally.

DR. SVENDSEN: Correct.

DR. FETELL: So has that changed?

DR. SVENDSEN: Yes, and no with the cells secreting GDNF. We always get cell survival effect, but they always retract back from the muscle a little bit, and we just don't know why.

DR. FETELL: That's why you want to do the dual transplants, yes.

DR. SVENDSEN: Exactly. That's why I think, ultimately, if ALS faithfully recapitulates the SOD1 rat model, which I don't think it will, because we're using sporadic patients, then we will need to do GDNF to the muscle. If, as I predict, ALS is a different mechanism, it's not an SOD1 disease, we don't know.

I mean, it could leave the GDNF alone and the spinal cord could maintain the functional connection or it might not. And so we'll only tell from that first patient trial.

DR. PORRECA: Thanks, Clive. We're running

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a little bit late, and we're going to have more time for discussion afterwards, so let's move on to the next lecture.

So it's a pleasure to introduce Allan Basbaum from UCSF, and Allan is going to continue in the theme of stem cells, and, in this case, more focused on pain, a very exciting topic.

Presentation – Allan Basbaum

DR. BASBAUM: Thank you. Thank you very much. I am learning a lot, which is great. It's the best thing I can say about any meeting, especially if you try to put it together with Frank. We have had a good time.

So I like to begin with this slide. I was editor-in-chief of Pain for 10 years, and the only good thing about being editor-in-chief is I got to pick the covers. So I thought, this is my legacy, and it's kind of fun. I'll mention a couple of these as we go through because they are somewhat relevant.

What I'm going to talk about is the use of transplants, and you heard this. And it was a

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great segue, so it's a good opportunity. And I'll talk not only about pain, but also a little bit at the end about itch. I have nothing to report -- I wish I did -- that is relevant to this at all.

So this is the kind of problem that we're dealing with. We're not trying to treat acute pain. I'm going to speak specifically about neuropathic pain, which is a pain produced associated with nerve injury, usually peripheral nerve injury.

This is a patient with complex regional pain syndrome. The individual probably has a minor nerve injury of the limb. And you can see that there's swelling, there's redness, there's ongoing pain, and incredible hypersensitivity. There's also a dystonic posture, which is something that's characteristic of this particular condition, very difficult to treat, doesn't respond to the traditional therapies such as opioids or NSAIDs. Clearly, something needs to be done.

This is, in a sense, one of my favorite covers from the journal. It's actually a patient

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who is a sculptor, who generated this sculpture of his foot, trying to illustrate what his problem is. And I think it's emotive, pretty dramatic, but it illustrates what the clinical problem is. And that is what the clinicians -- I am not a clinician, but I have seen patients, and it's a clear major unmet need.

So what are the contributors to neuropathic pain? And I could give a two-hour lecture on that quite easily. And there's a whole list. And everybody has their favorite mechanism. And I just put a few in here. And I'm not going to go through them at all because I'm going to focus specifically on the first, but there are issues of -- Frank likes to talk about descending facilitation mechanisms, microglial activation. Yves De Koninck has published some of the most elegant work on chloride changes, chloride gradient changes. Sprouting is another issue, sympathetics.

Let's just talk about GABAergic loss of inhibition, which I think is one of the major contributors, particularly the level of the spinal

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cord. And so I'm going to use this diagram in an illustrative way.

It's a half of a spinal cord. Here is an afferent fiber. They're all glutamatergic, provides input to the spinal cord, cell body, and dorsal root ganglion. Here's the output cells, that if this were an acute painful stimulus, the information would go to the brain and the patient would experience pain or the animal. I threw in a few inhibitory interneurons. The great majority, if not all of them, the superficial dorsal horn, are GABAergic. Some also contain glycine.

They're of course excitatory interneurons, but that's not the point of the topic today. And what we know is that when there's a peripheral nerve injury, usually a partial nerve injury, there is a loss of inhibition. In this case, I've illustrated as if there's a loss of, actually, the interneurons. That's controversial.

There is a paper from Clifford Woolf's group suggesting that you can lose about 20 percent of the cells. That's controversial, but what everyone

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agrees is that there is certainly decreased inhibition. And that could be reflected in measuring inhibitory potentials in the output cells, measure levels of the enzyme that synthesizes GABA. It's decreased.

So there's no question, in many ways, it's a seizure-like condition, where you have in a cortex -- in epilepsy, you have a loss of inhibition. And how you treat that, of course, is with anticonvulsants.

In this case, the loss of inhibition is associated with increased ongoing pain because of spontaneous pain that's not regulated, and hypersensitivity because the information coming in intact afferents is now on a hyperexcited spinal cord.

So how do you treat this? Just as in epilepsy, the standard treatment would be to use anticonvulsants. You're trying to increase inhibition, not necessarily GABA, but you're basically trying to increase inhibition. And any time a new anticonvulsant comes on the market, it's

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immediately put into patients with neuropathic pain because that's the source of the ones that have been effective so far. And the idea is, hopefully, that will reduce the pain.

This is a pharmacological approach that works in some patients. There are clearly many patients who do not respond, and the amount of relief they get is less than ideal. I would think people would agree.

So our approach is really to try to consider this more of a disease, and where the disease is a loss of cells or inhibition, and can we actually fix the problem, fix the neurological problem. And the approach that we're taking is to use cells, progenitor cells, from the cortex. Our initial studies from the embryonic mouse cortex to -- these are progenitors of GABAergic neurons, transplant them into spinal cord and see if you will repair the spinal cord.

The story begins with the work of John Rubenstein at UCSF. And John is a psychiatrist who studies the development of cortex. In this case,

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it's a mouse. You're looking at a mouse embryo. And what John is interested in is what is the origin of all different cell types in the cerebral cortex?

The relevant studies for our work was his demonstration that all of the GABAergic interneurons, inhibitory interneurons of the cortex, derive from a region of forebrain. The medial ganglionic eminence; that's MGE. And the MGE is the source of all cells in the cerebral cortex. The cells are born there, and then they will migrate, and they will turn into GABAergic interneurons.

It was this paper that turned us on to the approach. This is a paper in PNAS by Scott Baraban, along with other colleagues at UCSF. And what they did is they took mouse, 13.5 embryo. It was a GAD-GFP mouse, where the enzyme that synthesizes GABA was linked to GFP so that you could see green GABA progenitors.

They took those cells. They transplanted them in that time into a neonatal rat/mouse cortex.

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And then they followed them over time. And what they found is that the cells would take on properties, neurochemical properties characteristic of GABAergic interneurons. And they would look like interneurons, and they would distribute through the cortex. In fact, you can put them in occipital cortex, and they would populate the entire brain, just as the normal embryonic cells do.

When I saw this paper, it was interesting, but what was even more interesting with this paper is, they also transplanted the cells into a mouse with a potassium-channel defect. And this mouse with the potassium-channel defect had high propensity for seizures, spontaneous seizures.

So they transplanted into the neonatal mouse, and what they found is that the animal had significantly reduced seizures. That's what caught my attention, of course, because, as I said, I see to some extent that problem of neuropathic pain, at least at the level of spinal cord, has a loss of inhibition and a sense of seizure-like condition.

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So I went to John. I like telling this story because it's true. I asked John, "Do you think we could try it in spinal cord?" And he said, "Sure. You can try anything." But he says he didn't think it would work for a variety of reasons. Number one, we wanted to transplant it into adult and, at that time, we were only transplanting into neonatal. He thought that the adult might not accept the cells.

Also, these are destined to become cortical cells, not spinal cord cells, so he says they might not survive, wrong environment. And then, in the best case, even if they survived, they will probably migrate. And like E.T. come home, they'll actually go back to the cortex, because in the cortex, they will really move a long way.

So as I say, with that encouragement, we went ahead and tried it. And Joao Braz, a terrific fellow, is actually a virologist originally and now a terrific neurobiologist, tried it, transplanted into spinal cord. And the reason why I'm here is that it works.

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So the first question is can these cells -- now, we're taking from 13.5 embryo. They're progenitors. They are postmitotic. They are not yet neurons. And the question is, will they survive?

So here's the model. Here's our half a spinal cord again. We have an afferent. And I put in a few of the output yellow cells and then lots of inhibitory interneurons. What I told you is that when there's peripheral nerve injury, either lose the cells -- and I actually personally think that the major problem is loss of inhibition, not so much the cells.

Then we transplant. We started at about 50,000 cells. That's what we start with. We end up with maybe 10 percent of those cells. And to be honest, we don't know where they went. They might end up in CSF. They might get lost. They might die. We have looked at later for tunnel staining for apoptosis. We don't see it. It's possible they die very early on. We're not sure. And so we transplant them. And the question is, do they

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survive.
So this is what a transplant looks like at one day. You get a plug of cells. Here is the spinal cord. Here's the dorsal horn. The ventral horn would be here, the dorsal column, white matter.
So here's the plug of cells at one day and this is what it looks like at four weeks. The cells start to migrate, but they don't migrate very far, fortunately. They stay relatively close to the segment. They never go to the opposite side. They certainly don't go up the cervical cord.
This is an illustration. It's a sagittal section of spinal cord, caudal. This is the tail and here's the head of the animal. In this case, you can see there were four injections, and what I want to point out is, if you look, you can actually see what looks like axons. And I'll try to convince you that that's in fact what we're seeing. So these things will send out long processes, but the cells pretty much stay near to where you inject it.

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This is what a single cell can look like. They are very happy. They survive. They put out processes. And I will try to convince you that they really are integrated into a circuit.
Do they actually synthesize GABA and with an antibody against GABA? Here are some cells. These are the green cells, the progenitors. These are all terminals from the transplanted cells with an antibody against GABA. You can see that there clearly are GABA expressing.
Just for the record, we have recently transplanted cells from a VGAT mutant mouse that can't -- it can make GABA, but it can't store it in vesicles. And those cells survive, but they don't work, as you'll see later, but here are examples.
I'm just going through this part quickly. This part is published. Do they integrate? And I think this is really perhaps our biggest contribution because one of the major questions that was mentioned earlier is, are you generating a pump or is it actually integrating into the host circuit?

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So what we did in this case was just looking at primary afferents, but we've done it with other host cells. Over the years, we have generated a variety of tracer mice, where we can turn tracers on in the host and ask whether the tracer ends up in the transplanted cell or does the host talk to the trace, to the cells that contain the tracer.
The idea is that the question we're asking is does this cell, for example, talk to this cell, and do the transplanted cells talk to the host cells, interneurons and primary afferents, actually make synapses with the transplanted cells, which would suggest that they're really integrated.
So, as I said, we can turn a tracer on in the afferent, and it'll jump synapses. It's wheat germ agglutinin. And when we do that, we can look -- and just give you one example. Here's a cell where the tracer was turned on way out here in the dorsal ganglion, and it went into the cord -- a transplant that ends up in a cell in the cord.
Here is the transplant. So we turn the cells on, turn the tracer on, in a four-week-old

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transplant. Here are some cells. And if we go to high power, what you can see is here's the cell that receives the tracer from the afferent, jump the synapse, ended up in a post-synaptic cell in the dorsal horn. And you can see terminals from the transplant surrounding the cell.
Now, many people will look at this and say, "Wow. There are synapses there," but I was trained as an anatomist, and if you want to see synapses, you take it to the electron-microscopic level, which my colleague, Ida Llewellyn Smith, has now done. She comes to the lab twice a year.
Here's an example now, taking these transplanted cells to the EM level. Here is a synapse from the host, talking to a labeled cell body. This is a transplanted cell, so there's clear synapses. Here's a dendrite. People who know this would see this as almost certainly a primary afferent characteristic, scallop-shaped afferent terminal with vesicles. It's pre-synaptic to a host dendrite, pre-synaptic to the transplanted dendrite.

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This is an example of a terminal from the transplant that is now talking to the host. And most recently, we've actually done this. This is kind of tough. I honestly can't see it from here. There are black dots here, which is gold that marks GABA, just to illustrate that a labeled profile -- I hope you can see it, it's very fuzzy to me -- actually is GABA -- indeed, the terminal is GABA positive.

Here's an example, which was a bit surprising, that here's a dendrite from the transplant. And it is actually post-synaptic to a GABA terminal, which suggests that, in fact, inhibitory interneurons, host inhibitory interneurons, are really talking to the transplant.

Here's a better example. This is a terminal that has the gold particles, a gold marking, a GABAergic host terminal that is pre-synaptic to a dendrite that is expressing, so that the cell can even itself come under inhibitory control, so that it is integrating into the circuit. It receives afferent input, and it is controllable, if you

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will, by the host. So this transplant is really well integrated.

Is it actually functional? Well, very recently, with terrific post doc Alex Etlin, we've been doing sliced physiology in transplanted cells. Here's an example of a slice of spinal cord from an eight-week-old mouse. You can see the green cells. And then he's patching on to the cells. If you just depolarize them, the cells will fire.

Then more recently, using a variety of other electrical stimulation of attached dorsal route or more recently using channelrhodopsin that's been put into afferent fibers, into TRPV1 afferents, you can stimulate this and show that you can actually drive the transplant from stimulation of the afferent.

So the host is definitely talking to the circuit and, most interestingly, recently, we've made transplants from channelrhodopsin expressing mice. And then you can just stimulate the entire slice, stimulate all the cells with light, and you can show that the host cells are inhibited.

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So these are very functional. Another functional way of demonstrating this is to take, in this case, a four-week-old transplanted mouse. These are the cells. And then stimulate the paw of the animal and induce Fos expression, just a genetic marker of activity of populations of interneurons using antibody against the Fos protein. And every one of these red dots represents a nucleus that contains the Fos protein. And the question is, are they double-labeled? And the answer is, some of them are.

So these cells clearly were driven by activity in the periphery -- this is in a four-week-old animal -- and induced Fos in response to a stimulation of the afferent. So the transplanted cells are engaged, are integrated, and can be driven by peripheral activity.

The question, of course, is, so what? Can they actually influence the hypersensitivity that's characteristic of not only the patient, but more in the animal models that we use? And we discussed this yesterday, the complexity of the animal

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models. But we use a model of CRPS, or it's a model of mechanical hypersensitivity is a better description. And it's in this case the SNI, sciatic nerve injury model.

In the rodent, the sciatic nerve trifurcates to three branches. We cut two of the three branches and then isolate the tibial in this case. Other people do it by isolating the sural. And what you find out is, within 24 hours of this surgical procedure, the animals are extremely hypersensitive through the intact afferent. And you look at that and the question is, can the transplant mitigate the hypersensitivity produced by this.

This is the result that was published. So what we're plotting here is a normalized threshold on the Y axis. Both the control animals that receive either dead cells or medium start off at 100 percent. You injure. Threshold plummets. And then you transplant. This is all done blind.

The person transplanting does not test the animals. The person testing the animals doesn't

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know which cells have been put in or whether this is a control. And the code is only broken well after the experiment is done. The animals are killed. The cells or the distribution is monitored.

What you see is that this is the control animals where the animals are hypersensitive. And this will last for long periods of time. They don't recover. And the transplanted animals, over time, will normalize.

What's interesting is we never see them go above normal. We have done this many times now. This is the first example. We have repeated this in a Taxol model of hypersensitivity using four repeated injections of Taxol. The animals become very hypersensitive over all four limbs, but the transplant will only treat one of the limbs, wherever you transplant. And it'll treat both mechanical and heat hypersensitivity, which is characteristic of that model. So it's not unique just to this model.

We never see the animals go above. In other

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words, if we take a normal animal and transplant, the animals don't become analgesic. And this is an interesting issue, and we can talk about it in discussion. Our feeling is that the transplant is reestablishing a level of inhibition. In fact, we've monitored GAD levels. They normalize. They never go above normal. And I think this adjustment is what is critical to the function of the transplant.

So I will conclude at this point to say that, because of the nature of the integration, the extent of integration, this isn't just a transplant that's providing GABA as in a pump-like fashion. I do believe that the integration into the circuit is, in that sense, disease modifying to the extent that you can call this type of neuropathic pain condition a disease. Now, the neurological world, I know, doesn't like that term, but I think it's a reasonable description of the nature of this condition.

So where do we go from here? Now, some of you have seen this, but I just love it. It's very

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San Francisco.

(Laughter.)

DR. BASBAUM: This was a sign that was outside my daughter's home. There's a psychic that literally lives there and comes in next door in this restaurant. But due to unforeseen circumstances, I'm not sure this is the best psychic to go to. I love it.

(Laughter.)

DR. BASBAUM: Anyway, as I said, one of the things we've done is we're using other models, and it is very effective in the Taxol model. We're trying some others. The other thing we're interested in doing from a clinical perspective -- because we would like to think that this could be taken forward -- is transplanting into dorsal ganglion and trigeminal ganglion.

In particular, I'm most interested in trigeminal ganglion. I'm part of this advisory board looking at facial pains and particularly trigeminal neuralgia, which is a miserable condition. Many of you may be familiar with it.

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This is a characteristic picture of an individual with trigeminal neuralgia. The actual etiology is not clear. Some patients respond to carbamazepine. Some don't. Some will undergo a pretty drastic surgery, decompression surgery, to remove a blood vessel that may be affecting the trigeminal nerve, but in other cases, nothing works.

It seems plausible that this could be treated in the same fashion. We are transplanting it to nucleus caudalis, the dorsal horn of the medulla, in animal facial pain models. There is no good model of trigeminal neuralgia, unfortunately, that I know of in rodents or any animal for that matter.

But the question is, could you just transplant into the ganglion? And the answer is yes. Someone said, why would you? There's no synapses there. Well, that's true, but there are receptors. These cells make the receptors that are targets of GABA. And it's perfectly plausible to think that you could use this as a clinical target,

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readily accessible from a neurosurgical perspective.

This is an example of a transplant into the ganglion. These are green cells. And if you stain for GFAP, which will mark the satellite cells, there's no overlap. These are neurons. They express. They're NeuN positive, which is the same that we find in the spinal cord. I did mention that we find about 80 to 85 percent of transplanted cells are NeuN positive. We don't see oligo or astrocyte markers in these cells.

This is just to illustrate that we're doing these studies now with Xiaobing Yu, who is an anesthesiologist pain fellow in the lab. And it's been a bear and a mouse, but this is just a mouse that's been injected, in this case, with dye just to illustrate here, the trigeminal ganglion. It's done stereotaxically. This is just to illustrate it can be done. We're now recording before transplanting.

These are some green cells that are in the trigeminal ganglion. It was an early example.

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Xiaobing is getting a lot better at it. So we are trying to see whether this can be effective in a variety of animal models of pain.

A little segue, chronic itch. This paper caught our attention from Sarah Ross, who is at University of Pittsburgh. When she was working with Mike Greenberg at Harvard, they found that a BHLHB5 mutant mouse -- this is a transcription factor that expresses and is necessary for the survival of a population of GABAergic interneurons in the dorsal horn of the spinal cord.

You lose many of them in this mouse. And what they found is, after about two months, six to eight weeks, actually, the animals start to develop this spontaneous syndrome of intense scratching, terrible scratching.

Their hypothesis is as follows, that under normal conditions, just like in the pain, it looks like a gate control theory model, there is an afferent input that drives the itch circuit like a pain circuit, but there's also a feet-forward inhibition that regulates the output of the cell to

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keep it under control. And in this mouse, where you lose these cells, the animals have heightened itch.

So I wrote to Sarah and I said, "Do you think we could try this?" The animals are not healthy animals, so it was tough, but she sent us the mice, and we've now -- Joao has now transplanted, and we have a paper in JCI that's just been accepted.

This is the spontaneous scratching that occurs, so the animals are just scratching. And so somebody is now monitoring. They don't know which side. The animals will scratch on both sides and usually have symmetrical scratching. The individual who is monitoring this doesn't know where the transplant was. Within two weeks, we actually start to see a significant decrease. You can't go much longer because the control animals have to be euthanized.

This is an example, and it really is characteristic of an animal in following it out. This is what it looked like at about six weeks of

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age. The animals were transplanted and follow the animal out. And then by this time, literally, the hair is starting to grow back.

When we saw this initially, I called Sarah, and I said, "Have you ever seen spontaneous recovery in these animals?" And she's done a lot, and she's never, absolutely never. They always have to euthanize them. And we've done this many times at back of the neck, depending on where the scratching occurs. It's not predictable. And we transplant where the scratching -- the part of the appropriate dermatome spinal segment, and you can treat these animals.

Finally, this is where we'd like to go. We actually did try fetal transplants. We went to 16-week and 13-week fetus, tried to get MGE-equivalent cells from human fetus, and we transplanted. And the cells survive in the mouse. These are in SCID mice, but we never got neurons. All we got was oligos, beautiful oligos, lots of them. And I'd like to learn more about that because I'm not a stem cell person, but we're

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getting these cells from our stem cell folks at UCSF.

But most recently, we have been using human embryonic stem cells that are modified to become GABAergic neurons. And the answer is that they work. They will survive. This is now transplanted to SCID mice. This is now six months post-transplant. These are markers. I noticed the similar markers. It's a stem cell marker to identify the cells. This just illustrates doublecortin and Ki-67 will mark cells that are dividing, and we don't see that. By six months, we no longer see that these are dividing.

We don't see tumors. This is, as you said, one of the big concerns. We have not seen tumors in the mice. So the cells, they become, they take, they grow, they move a lot more than the medial ganglionic eminent cells; don't go to the other side. They'll go to white matter. We don't see them going to cervical cord, but they are definitely moving more. And by six months, they take on -- this is staining for GABA, bunches of

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cells. We've got NPY-positive cells, different co-markers. So the cells do take on the properties of the inhibitory interneurons, a smaller percentage than the MGE cells for sure.

I'm not going to show the data, but I'll just tell you, can it do anything in that same model of nerve injury. And the answer is yes. After six months, it takes a long time. We can get about 50 percent recovery of threshold. We never get anything like the MGE cells, where we can return it back to normal.

We get about 50 percent recovery, but it takes a very long time. And the real reason, unfortunately -- well, not unfortunately; it's the nature of the cells, my understanding. And it's a fact; we tried it. If you transplant the cells after they've been differentiated, if you will, or driven into a GABAergic phenotype in vitro, and then transplant them, they don't take.

So you need to feed them, and then transplant them, and then keep your fingers, and it takes a long time. So this is one of the

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limitations when you're working with mice.

I assume the stem cell people are going to work out ways to get these things to grow a little bit better. We are also doing the same approach you described of using Lenti and doing this mostly in the MGE cells to turn different elements, different things on in the transplanted cells.

For example, enkephalin, to release enkephalin in a circuit fashion rather than pouring it on the spinal cord, we think that we can enhance the utility of the GABAergic component by having the cells generate something else. So we're excited, and this is where we are continuing. We would love to be able to see this taken all the way. There's a lot of hurdles before. But that's where we are now.

In conclusion, there are a lot of things that cause neuropathic pain, but I think thinking of it as a neurological disease that can be repaired is something to consider. And with that, I'll introduce you. It's not as cute as your slide, but I have a lot of cute people in the lab.

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For the record, what this says here is, "Pain is in the brain," and that's because Frank is sitting in the front row. And I know that pain is in the brain. And this is Joao Braz, who did most of the transplant work. Alex did the slice physiology. Xidao did a lot of the behavior, the blind behavior work. And Reza Sharif-Naeini, who is now on the faculty at McGill, did some of the early work with Joao. Ida did the electron microscopy. And most recently, I've been working with the stem cell folks to try to get the embryonic stem cells to work.

We do want to move to iPS cells. We're not there yet. Thanks very much.

(Applause.)

DR. PORRECA: Thanks, Allan. Very exciting. Any questions? Andrew?

DR. RICE: Thank you. As I was listening, I was just wondering what kind of clinical trials we would think about in the ethos of this meeting. And you've partially already mentioned this in your reference to other polyneuropathies, but I was

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wondering, we're increasingly recognizing the heterogeneity of neuropathic pain presentations in the clinic and even now in animal models. And you referred to some of the other animal models. But many of the polyneuropathies, diabetes, HIV, drug-induced, are characterized largely by sensory loss, the diabetic neuropathies in the clinic. And the central core of this is the loss of GABAergic cells in the DRG associated, I presume.

DR. BASBAUM: In the cord.

DR. RICE: It's in the cord, with traumatic nerve injury. And I just wondered to what extent that is shared with the other models you've mentioned like the trigeminal ganglion? And obviously, it's not the cord, but also the Taxol neuropathies. Do you see a similar loss of GABAergic cells?

DR. BASBAUM: No. No. In the case of the Taxol, we don't, but what you do see is a decreased GAD function. We have never seen loss of cells. I should point that out. The literature has reported loss of cells. But you do see decreased GAD in

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these models. It's true that most of the models we use are traumatic.

Now, one of the key questions is, if we're right in our hypothesis that what the transplants are doing is readjusting, normalizing the level of inhibition, then I think it would be effective regardless. You don't have to lose cells. For whatever reason, you get decreased inhibitory regulation, and the idea is to re-establish that.

There's a variety of other interesting aspects. These cells retain their cortical phenotype. So cortical GABAergic cells have a different neurochemistry from those in the cord. And most characteristic is -- somatostatin is characteristic of many of the cells, and the cord, somatostatin, doesn't co-occur. In fact, it's in a different population. It's not in GABA cells. These stay -- many of them are still somatostatin positive.

So the environment is not driving them into a spinal cord phenotype. They're just sitting there. And by the way, the electron microscopy

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changes over time. Ida has looked at four weeks and looked a six months. The number of axial somatic synapses at four weeks is much greater than at six months.

So the integration is occurring over time. The system is unbelievably plastic. And my take-home lesson is the system is remarkably plastic. And I really do believe it's adjusting to a level of inhibition and that it can be useful under any circumstances.

In the same case, why does gabapentin work, at least to some extent, in so many of these heterogeneous polyneuropathies and conditions, even though they have a different etiology, adjusting the level of inhibition, can help.

DR. MCMAHON: That's very interesting from lots of points of view. One question is whether the effects are specific on pain or whether you have effects on locomotion, where the animals are kind of --

DR. BASBAUM: We've popped them on a rotarod, and it doesn't make any difference. Now,

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we haven't targeted ventral horn. And once in a while, you do miss, but Joao is getting pretty good at making those injections to dorsal horn. So if we target specifically a ventral horn, would it make a difference? I don't know.

DR. MCMAHON: My main interest is what the mechanism is. And so you raised the question of whether it's just passage secretion or whether it's a neuronal integration. Some of your early behavioral data, so in both the itch and in the spared nerve injury, you're seeing effects from a few days or a couple of weeks.

I assume, at that stage, there wouldn't have been a lot of integration then. So early on, they may be pump-like --

DR. BASBAUM: Good point.

DR. MCMAHON: -- as you said, with time, it looks as if they become more stabilized. But that is critical because maybe you could just use some astrocytes and make them make GABA. And we'd have one quality-controlled line and save everyone making the weak cells.

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DR. BASBAUM: Absolutely, yes.
DR. MCMAHON: So you haven't tried, either, to do that, to deliver GABA from a non-excitable cell or maybe I was wondering whether there was an easy way of making neurons non-excitable so that you could turn them into pump-only for a direct comparison.
DR. BASBAUM: If I wanted to do that experiment. I really don't want to do that experiment because my philosophy is that the pump-only would be equivalent to administering the drug intrathecally, which is associated with side effects.
DR. MCMAHON: Yes.
DR. BASBAUM: And I think the reason why the therapeutic window might be better with this is that it is being delivered in a circuit fashion. Remember when I said, if we transplant from the VGAT mutants, the cells take beautifully, but they don't do anything. So you need GABA for sure to come out of these cells.
I don't know. I could be wrong. A pump

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might work just fine, but somehow it seems to me that the integration is key.
DR. MCMAHON: In the last talk, though, we heard that the location might be as important as integration. Just having the neurons in the neutrophil delivering loci, that may overcome.
DR. BASBAUM: Fair enough.
DR. MCMAHON: It's obviously just an interesting question to know what the contribution is from these distinct mechanisms.
DR. BASBAUM: No. I completely agree with you, but I don't have CERN money.
(Laughter.)
DR. BASBAUM: No. We actually applied for it and didn't get it because we were too late. We got into this too late, and now, all they care about is they want a therapy. No. They were very -- they said this is really cool, but as long as you're using mice, we're not interested, seriously.
DR. PORRECA: Allan, just a follow-up question, you were talking about different types of

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cells, some of which have this GABA phenotype, as you are presented, but how do you know the effect is from GABA? The cells may be releasing other factors --
DR. BASBAUM: No. Sure. No, completely true. Did you review our grant?
(Laughter.)
DR. BASBAUM: That was one of the questions. The other one, you've got to do physiology. You've got to do ultrastructures, so that's why we're doing it all.
No. It's a very important question. That's why we did the VGAT mutant mice, where they are identical cells, except they can't store GABA. So they have no GABA. These animals will not survive because they are just born and they'd seize all the time. But we're taking embryonic cells, so we can take from the 13.5 embryo, and they don't work, basically. The cells integrate beautifully. They don't do anything.
So I think that it looks pretty good that GABA is at least necessary. It may not be

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sufficient. We don't know that.
Now it's my pleasure to introduce Joe Glorioso, who is not only a terrific scientist, but a colleague and friend. Joe trained, I think, originally in Louisiana State, and then went to Michigan, and is now -- well, he was professor and chair of -- not microbiology --
DR. GLORIOSO: Molecular genetics.
DR. BASBAUM: -- molecular genetics.
DR. GLORIOSO: Twenty years is enough.
DR. BASBAUM: Twenty years is enough. I'm chair of the department. I've been 15. I saw it, and I got worried when I saw he was 20 years -- at University of Pittsburgh.
Joe is really one of the leaders, if not "the" leader, in the development of gene therapy approaches, particularly using viral vectors both in animal models and definitely an interest in taking this to humans. And the work is terrific, and I look forward to hearing his story. Welcome.
Presentation – Joseph Glorioso
DR. GLORIOSO: Thank you for that kind

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introduction.

Well, I have to say, it's been a great meeting. I really have learned some interesting things. So I'm pleased to have the opportunity to tell you about some of the new work that we're doing.

I have two disclosures to make. One of those is I'm a founding partner of Switch Bio, which is a company we just began, and also NuvoVec srl, which is an Italian company.

So this is the important biology of herpes simplex, and this is why we got into this, to treat pain. The virus naturally is neurotropic, meaning that it only establishes latency in sensory neurons. It can also establish latency in the brain, but it can't reactivate it. It usually doesn't reactivate.

The virus is transmitted by direct contact with the lesion, and it goes through the replication cycle. New particles are made, which, as you can see, they are transported retrogradely down axons. And they can go very long distance,

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and it's a very efficient process. The virus establishes latency in the nerve cell body.

The lytic genes are inactivated and, rather, you have an activation of this latency-associated transcript, which is a large messenger RNA that's non-coding. There is an interesting intron that's spliced from this, that's highly stable. It's associated with the nucleolus. You can rarely detect it by in situ hybridization.

This is the hallmark of latency. The natural virus can reactivate usually as a result of nerve cell damage. In rare cases, it can go back and cause encephalitis. For doing gene transfer to sensory nerves, we want to use a virus that's highly defective.

It can establish a latent light condition and serve as a platform for expression of trans genes. The virus cannot reactivate or cause lytic infection. Again, you can detect gene products in the nerve cell body.

So what I'm going to focus on today is treatment of post-herpetic neuralgia as a good

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target, we think, for this kind of therapy. The post-herpetic neuralgia is caused by varicella zoster virus. It's the virus that causes chickenpox, usually occurs at an early age.

It establishes latency in sensory nerves just like HSV-1 and HSV-2, usually as people age. There's a recurrence of about 20 percent where the virus reactivates and causes herpes zoster, referred to as shingles. And this is an important problem because it causes pain.

Ninety percent of these patients are given pain meds. About 30 percent have a long-lasting or chronic pain lasting beyond 90 days, and this is referred to as PHN. There is an estimated about 200,000 new cases in the U.S. every year, and the principal complaint is allodynia.

So one of the tricky things about doing pain modeling research is to find a good model. This is the one that's available. It was a model established by Fleetwood Walker in 1999. What he did was injected VZV-infected cells. This is the vaccine strain P-Oka. The cell lines is MeWo.

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They injected it in the footpad of rats.

The reason we used infected cells as opposed to virus is because these viruses are very hard to purify from infected cells, and everybody in the field that works with VZV uses infected cells. And we looked for mechanical allodynia and pain responses due to heat.

So this is the general approach, that we injected infected cells about 2 times 10 to the 5th into the footpad and into the right-hind paw, and then we measured pain responses over a period of about nine weeks.

So these animals both develop mechanical allodynias. You can see here as well as in a paw withdrawal latency model, latency response. They also developed thermal hyperalgesia.

So in addition, we can see that the virus enters by leaving these infected cells, going up by axonal transport to the nerve cell bodies. You can detect viral antigens. And for example, this is an antibody specific for one of the metered early proteins, 62, and you don't see this with

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uninfected cells.
So one of the first set of experiments we did was to use preproenkephalin as an analgesic. We have a lot of experience with that in the first of these experiments back in the mid '90s. Preproenkephalin, as you know, is processed into these opiate peptides, and they combine to both delta and mu receptors.
So this is the general approach, that we inject virus into the skin. And the virus can enter these nerve terminals, both the C fiber and A delta fibers, be transported back to the nerve cell body. And here, the virus can produce in the dorsal horn and the spinal cord enkephalin, as shown here. Enkephalin then can bind back to its receptors both in the primary afferent as well as the second-order neurons.
Enkephalin is also made by descending neurons as well as GABA, and this also can be analgesic. However, you can't control when these products are going to be made in natural occurrences. So the idea is to produce this

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product locally and treat pain at its source.
So this is the general approach, to treat PHN with enkephalin vectors. And what happens here is we inject the infected cells. Several weeks later, the animals begin to develop pain responses. And we inject the vector about two weeks after the primary infection. And the virus either expresses enkephalin or just a reporter gene.
So this is an example of the kind of data you get with mechanical allodynia, that the animals initially, if they are injected just with cells without virus infection or plus the control virus, you don't get any allodynic response. However, if you inject animals with the pOka-infected cells with PBS or control virus, you get this very strong allodynic response, which you can reverse by infection and expression of enkephalin.
Now, we also ask the question whether you can preempt this response, thinking that maybe even in a prodromal situation, you could treat patients. And so we injected the vector, making enkephalin our control. And a week later, we injected the

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cells that were going to cause pain in these animals.
You can indeed block pain responses. So for example, in animals that got enkephalin-producing virus plus the pOka-infected cells, we didn't see any evidence of the development of allodynia.
So while this looks pretty good, there are some limitations, we feel, in this approach. And that is, enkephalin therapy is transient. And if you want to keep it on longer, you have to do repeat dosing, which I think over months is not going to be feasible in patients.
You can achieve long-term expression of enkephalin by using the latency promoter system, but this might cause tolerance or some other unwanted side effects. And how do you turn off this therapy if you no longer need it? So our view was that we needed a solution, which involved some kind of regulated gene therapy, and that's what I'm going to talk about.
So a few years ago, we had the idea that if you used a chloride channel -- in this case, it's

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the glycine receptor, which is not found in sensory nerves, it's only found in the central nervous system -- we might be able to induce silencing in these cells by delivery of glycine. And it turns out you can only use the alpha subunit because this has turned out to be pretty fortuitous because this product is spread up and down the axon. Rather than hyper-polarization, we think probably it's silencing the neurons by depolarizing the cells, and up and down the axon, blocking the development of an action potential.
We published some experiments fairly recently in which we used the glycine receptor, the alpha subunit, and injected glycine into these animals, and we could in fact silence these neurons and block pain in a lot of different kinds of models.
We tried this also in a bladder model, which worked very well, and we gave the glycine systemically. However, the treatment window was very narrow. In other words, as you increase the amount of glycine being given -- we inject their

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IP, for example -- you begin to see effects in the central nervous system, so we needed some solution to that problem.

So about that same time, Joe Lynch's lab in Australia published this nice study where he made mutations in the glycine receptor that made it resistant to glycine activation, but rather was strongly activated by the drug ivermectin, which is an antihelminthic drug that's been approved for patient use as well as in animals for a number of years.

So we tested this system, and what we did is we injected either the normal receptor that's responsive to glycine or the mutant receptor that was responsive to ivermectin, and we tested this in a standard pain model.

What you see here is in a paw withdrawal latency test, this is what the average responses are like, that if you inject the virus that's making this a normal receptor, it's highly responsive to glycine and you change for paw withdrawal latency. But it's not responsive to

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ivermectin, and we did these experiments in two and three weeks.

The opposite was true if you inject a vector that's making the mutant receptor, that it's highly responsive to ivermectin and not responsive to glycine. We saw this. We got this data about 10 months ago, and we were very excited because this, for the first time, would allow us to target a specific receptor, and we could give this drug systemically.

So we tested this in a rat PHN model. And what we did, again, was, we injected cells. And after two weeks, we injected the vector either making the normal glycine receptor or the mutant glycine receptor. And I'm just going to show you the data with the mutant.

So in this experiment, this is mechanical allodynia here and thermal hyperalgesia here. And what we did is we injected either a control vector that made just GFP, or a vector that made the wild-type glycine receptor, or the mutant glycine receptor. And here we got a strong response,

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reversing mechanical allodynia by injecting the ivermectin.

We did a dosing experiment, so when we got down, even as far down as 10 nanomolar, we were able to show an effect. And it turns out that if you use the glycine receptor, you can see effects out to 25 millimolars. So this is a very sensitive way to activate this receptor.

The experiment was done by injecting the drug into the footpad, where we injected the virus. And we're going to test very soon systemic delivery and see how well that works. We've got similar results with the thermal responses.

So we want to further develop this regulated nerve silencing because the use of this mutant glycine receptor is attractive. However, what about off-target effects? And since we're silencing every neuron where this product is being expressed, if it's expressed in neurons where we don't want silencing, such as neurons involving touch or proprioception, then we want to avoid those neurons. And so we started working with

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targeting viruses to the peripheral nervous system.

Now, there are a couple of ways that we are using this targeting strategy, and these are the two classes or subpopulations of neurons that we're after. Unmyelinated C fibers are most commonly thought of as being involved in chronic pain responses. And these can either be peptidergic or non-peptidergic.

So we used promoters that would give us transcriptional silencing, either for example N of 200, or the NPY promoter, or TRPV1, CGRP, and so on, and see if we can get expression of the receptor in the appropriate cell population.

Now, we have also done a lot of work with targeting the virus, which I won't have time to discuss. It's a pretty involved story. But if you knock out the virus's ability to recognize its cognate receptors by mutagenesis of a protein called glycoprotein D and fuse it with a pre-pro NGF or GDNF, you can target it to these two different receptors efficiently. So we haven't tested the combination of transcriptional and

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transductional targeting, but I think we're going to try that in the near term.

So this is the experiment where we introduced these recombinant promoters into the virus genome. In between these two insulator are two boundary elements that the virus naturally has. And we remove the latency promoter and replaced it with a tissue-specific promoter driving a transgene, mCherry. And this is just the series of promoters that I just told you about.

So I'm going to show you just examples of two of these. This is the GCRP promoter driving mCherry. This was done in a bladder model. And this particular ganglion, we stained with an antibody to GCRP, and we got about 65 positive cells.

Viruses that infected these cells that were expressing mCherry, using the GCRP promoter, found out that was pretty good overlap between those positive cells and those cells which were activating mCherry by this tissue-specific promoter. To use another promoter, which is

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non-specific, such as CMV, you get good expression, but very little overlap between the two types of cells.

This is an example with the TRPV1 promoter driving mCherry. This is in the rat footpad model. These are the numbers of TRPV1-positive cells that we saw. About 40 of these were M-cherry specific. They were driven by the TRPV1 promoter. And again, there was pretty good overlap in this particular experiment, 83 percent between the two. And for TRPV1 and mCherry expressed from the CMV promoter, again, there was not very good overlap.

So one strategy to use the mutant glycine receptor, then, is to silence TRPV1-positive cells because these are normally thought to be involved in chronic pain where TRPV1 itself is involved in, say, allodynia. It's not clear. But we might be able to enhance the specificity in certainly efficacy.

A second strategy would be targeting TRPV1 receptor by the expression of an antagonist. So we set out to find antagonists of TRPV1. TRPV1 is a

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tetramer that is activated by phosphorylation using PKC and other phosphorylating events, also in the inflammatory soup can activate this particular receptor, sensitize it, as well as asset. It can be down-regulated or desensitized by calcium-dependent phosphatases like calmodulin.

So what we were looking for is an antagonist which would block the activation of TRPV1. So what we did was to use the virus system, and we expressed two copies of TRPV1 in this virus. We had to use two because we found that mutations would occur that would give us false-positives, which didn't occur if we used two copies of the gene.

In the joint region of the virus -- this is a region that flanks the unique long and unique short portion of the genome -- we replace this with a library of expressed genes from PC12 cells after activation with NGF. And we analyzed this library by DNA microarray and found that we had about 15,000 unique genes. So this represents a large number of genes coming from these cell types.

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So this is the general strategy that when you infect with the virus that makes TRPV1 and activate it with capsaicin, calcium floods into the cell. You get osmotic destabilization. The cells die, and you don't get any virus plaques as a result.

However, if you infect with a TRPV1 virus that also expresses the gene that inactivates or blocks the activation of TRPV1, then many of these cells survive. You can get plaque formation. You can isolate these plaques, clone them in that way, sequence the insert, remark the virus, and retest it, and look for ones that are specific for blocking the activation of TRPV1.

For these experiments, we also used as a positive control a dominant negative form of TRPV1, which is called poreless, which had the transmembering region deleted, so when it makes heterodimers, inactivates TRPV1.

So in this experiment, if you infect cells and don't treat with capsaicin, with the vector, and it doesn't contain any antagonists, you get

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absolutely no virus being made. However, if that cell expresses one of the genes that we found, common phosphatase, PP1-alpha, compared with poreless, we've got very good rescue of virus growth.

So by looking at about 500,000 plaques, we've picked up about 50 genes which could antagonize TRPV1, and this was the most potent one we've found.

We could also show that, in rat fetal neurons on infection, using Fura-2 assays for calcium uptake, that both PP1-alpha and poreless, compared to the control vector, would block the uptake of calcium.

We tested this vector in a number of model systems. This is showing you one with a heat ramp, where we changed the temperature from 30 degrees up to about 44 degrees over a period of 15 minutes and looked at the response, the heat-related responses, pain-related responses in these animals, which for example they would curl up their foot and lift it or bite and lick at it. When you get to about 43

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degrees, then you start to see this TRPV1 receptor being turned on.

However, you can really exaggerate this response by treating with capsaicin. And you can see, with the control virus, you've got very little effect on the development of this pain response. However, PP1-alpha, as well as poreless, gave you a dramatic difference. And we were shocked to see how impervious these animals were to pain up to 45 degrees.

So we have also used this in the PHN model, and I'll show you the data from a couple of these experiments. What we did is we injected the VZV-infected cells two weeks later. We infected these animals with a PP1-alpha or a control vector. And we measured pain responses over a period of weeks.

What we saw was that in the case of the GFP-expressing vector, these animals rapidly developed mechanical allodynia. However, the vector that expressed PP1-alpha, it reversed this process, and it looked like a normal animal.

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The same is true with our paw withdrawal latency model, that the GFP didn't have any effect, whereas the vector that's making PP1-alpha had a dramatic effect on heat sensitivity.

I'm not going to show you all the data, but we've tested these in cold models. We have tested these in the formal and footpad test, where different receptors are known to be involved, and this product has no effect.

So to summarize, then, we think that gene therapy for PHN is feasible, and it should be effective, but we really need human trials to validate efficacy. Regulated gene silencing is, I think, a good strategy because it ignores essentially what the cause of pain is, but rather just silences those neurons, which are involved in pain.

I have to say that, at least if you inject ivermectin locally, the animals begin to lose the pain response within seconds, and it lasts for about three or four hours. So this particular drug has a good half-life in humans. It's about

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16 hours. And so it's a very benign drug. It doesn't go into the central nervous system. So it may be a good first step at trying to use the nerve silencing technology for blocking pain.

But we think that targeting subpopulations is going to be important, and it may be even possible that we can use antagonists, like the PP1-alpha story that I just told you about, as an additional therapeutic strategy.

I didn't say anything about the mechanism, but PP1-alpha has a lot of effects in cells, as you know, but it does dephosphorylate TRPV1. And it dephosphorylates R2, and probably has an effect on HDACs as an HDAC inhibitor.

So there are a number of people involved in this work. A couple of people I want to mention in particular, Bill Goins and Kip Kinchington, and Jean-Marc Guedon are the people that developed the PHN model and did all the PHN work that I'm showing you; Mark Doyal, who is an MSTP student that's worked on the transcriptional targeting methods. Bonnie Reinhart was the person that's done the

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targeting by modifying the virus infectivity. And Yoshitaka Miyagawa is our critical person in engineering the viruses. We have a whole new stable of engineered viruses that I didn't have time to show you. Thank you.

(Applause.)

DR. PORRECA: Some questions for Joe?

DR. BASBAUM: Joe, thank you. The thing I must say is, I was very surprised. I didn't know that promoters for these genes had actually been identified. Where did you get them?

DR. GLORIOSO: Yes. I don't have that on the tip of my tongue, but I can tell you what they are. There's been some publications for these different promoters, and we're using a fairly large piece of DNA for expression, so we have added additional sequences.

One of the advantages of HSV is, this new class of vectors, which I really haven't told you anything about, we can put up to 40 kilobases of foreign sequences into. And so we can put very complicated or large genes or complex promoter

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constructs, and I think that's going to be an advantage here.

DR. BASBAUM: If you inject the ivermectin in the opposite pod, does it do anything?

DR. GLORIOSO: No.

DR. BASBAUM: So it's not a systemic effect.

DR. GLORIOSO: It's not a systemic effect, but we're hoping that -- we haven't tried it systemically yet. So we're hoping that we can use it systemically. And ivermectin is used as a cream and it's used in a lot of different ways, so we will try to do that.

DR. BASBAUM: I apologize. I forgot I was chairing this session. That's why Brian gave me the microphone.

Any other questions? Andrew?

DR. RICE: Just a comment about the model -- and it may be a little -- well, it is pedantic, but it is important for thinking about how we translate this to the clinic.

First of all, I'd certainly echo your view that it's absolutely vital to have expert

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virologists working with you on this model, and Kip Kinchington is obviously well known in that field.

But I think to call it a model of post-herpetic neuralgia is pushing it too far, and we've probably made the same mistake, too. It's a model of acute zoster infection and the pain that occurs with that. And while it may share features of post-herpetic neuralgia, I think it's important to bear that in mind because, when we start to try and think about translating it into clinical trials, that may actually be important.

DR. GLORIOSO: Yes. This is an acute model, and we don't have a virus reactivation model to look at that. That's a good point.

DR. BASBAUM: Do you have evidence that the released enkephalin is actually acting on the primary afferent or is that just a hypothesis?

DR. GLORIOSO: It's a hypothesis because enkephalin is actually released into the skin as well. And it's released into the spinal cord. So I don't think it goes very far. I mean, some people -- we were thinking earlier, well, it may be

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a bystander effect, but I doubt that's really true. So I'm thinking that it's released locally and presumably can bind to its normal receptors.

DR. BASBAUM: It looks encouraging.

We should take a break. Then Toto will give us a talk, and then we'll have time for lots of questions. Thanks very much, Joe.

(Applause.)

(Whereupon, a brief recess was taken.)

DR. BASBAUM: If everyone can please grab a seat, so we can get started. So it's been a morning of somewhat esoteric approaches. And the next talk is not quite esoteric, but definitely unique, and we're in for a treat. I'd like to introduce Toto Olivera. Now, Toto grew up in the Philippines, where he I think got introduced as a kid to cone snails. And we're going to hear a lot about cone snails.

I think he did his undergraduate work in the Philippines and then went to Cal Tech, where he got his PhD. And after work at Stanford, he went to Salt Lake, where he's been for a long time as a

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professor -- not forever -- a professor of biology and has demonstrated the incredible utility of the products of these cone snails. And we will hear about this in a variety of conditions, but particularly in potentially the management of pain. So it's really a pleasure. Toto, welcome.

Presentation – Baldomero (Toto) Olivera

DR. OLIVERA: So I'd like to thank Allan and Frank for inviting me here. And I'd like to start out by just reviewing both the biology of cone snails and some of the older work that we did. But let me start out by saying that I have no disclosures, and I wasn't able to e-mail my presentation because there are lots of movies. So we ended up with no title slide and no disclosure slide.

But let me tell you about the cone snails. There are about 700 species of cone snails and all of them are venomous. And they are useful in that each species is quite specialized. So this species will only eat fish. This species will only eat certain types of marine worms. And this species

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will only eat certain types of snails.

As you will see, our cone snails are pretty good neuropharmacologists. And so what I'd like to do is not just tell you about the individual toxins that have applications to pain, but also what we've learned about the philosophy of how this neuropharmacological system has evolved because we believe that there are some applications of the general principles that the cone snails have evolved in practicing neuropharmacology.

So some of these snails are lethal to people. This one causes a fatality rate of 70 percent in the absence of medical intervention. And so we started long ago to ask the question, why is this snail able to kill people? And the answer is that there are two components in the venom that are paralytic. One of them blocks the nicotinic acetylcholine receptor, the post-synaptic terminus of the neuromuscular junction, just like cobra toxin or alpha bungarotoxin, while the other essentially wipes out action potentials in muscle by blocking the same site that tetrodotoxin blocks

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in this voltage-gated sodium channel.

So the sting of this snail is deadly because it's equivalent to being bitten by a cobra and eating a lethal dose of pufferfish at the same time. And that accounts for the 70 percent fatality rate.

Now, the unusual thing about the venom components is that they're all small peptides, most of them, highly cross-linked by disulfide bonds. And they have post-translationally modified amino acids very often.

So since we answered the question we first wanted to a long time ago, why am I still working on cone snails, the reason is really, an undergraduate at the University of Utah made a key discovery. So if you take the venom of that conus geographus and separate the venom components according to size, you can separate out peptides that are about 10 amino acids from those that are about 20 amino acids. And if you take this fraction and separate the peptides in that fraction, you can see there are lots of peptides.

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Only a few of them were paralytic using the assay that we had developed, which was IP injection into mice. Then this kid came along -- his name was Craig Clark -- and as is typical of 18-year-old kids, said, "Well, you're doing your assay all wrong. You shouldn't be injecting IP. You should be injecting ICV or intracranially." And I wasn't persuaded, but I think the nice thing about being at the university is that the students do what they want, not what we tell them.

(Laughter.)

DR. OLIVERA: So luckily for us, Craig did his experiment anyway. And so what I am going to show you is what he found by injecting this particular fraction of the venom into the brain of a mouse.

What he found was the paralytic toxins of course we knew about, but this peptide makes mice jump and twist as they are jumping. This peptide made mice uncoordinated. This major peak put mice to sleep. They'd be asleep for 12 to 24 hours, and then they'd wake up and be perfectly fine. But

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that was only true if the mice were under three weeks of age. If they were over three weeks of age, instead of going to sleep, they'd climb their cages constantly.

This made them drag their back legs. This peptide made them run around in circles. This made them swing their heads back and forth. This made them kick on their back and scratch. This made them depressed. This one caused convulsions. This one caused scratching, and there are a few that didn't do anything.

But as you can see, the remarkable result was that there was a behavioral phenotype that was induced when each component was injected directly into the central nervous system.

So this experiment of Craig told us that the venom of these snails was not a mixture of a few paralytic toxins, but was rather this very complex pharmacological brew of small peptides.

So all of these are just the peptides. There are about 20 amino acids. And then there are three other major fractions. And so you can see

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that the diversity in the venom is probably about 80 or 100 peptides that all seem to be biologically active.

So at the time, we had a whole troop of undergraduates coming in. They could choose any snail they wanted, and using Craig's assay, they could follow the activity of any peptide that they wanted. And their job was to purify the peptide that caused the behavioral phenotype until we knew the sequence of the peptide. Then we could synthesize it, and that way, we could continue to follow it up.

So this guy, again, an 18-year-old kid at the time, he chose conus magus, and he decided to purify an activity that he called the shaker peptide because it induced tremors when injected intracranially into mice.

The reason I am telling you this is because this peptide that Michael purified and characterized is today an approved drug for intractable pain. It's called Prialt. We called it Omega Conotoxin MVIIA. And so this was approved

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in 2004. And what I'd like to do is retrospectively tell you what we've learned about the development of Prialt.

So why did the snail evolve this peptide in the first place? Well, it's a fish-hunting cone snail. And in fact, of course, what's targeted is the neuromuscular circuitry. And in order for a fish to be able to swim, then of course an action potential has to cross a neuromuscular junction.

If we zero in into a signal vutron [ph], then we can see that the key event is when the electrical signal reaches the end of the neuromuscular junction, voltage-gated calcium channels needed to open. And when they open, of course, that causes neurotransmitter release. And that's necessary to get the signal across the synapse.

So of course, once acetylcholine is released, then it binds to the acetylcholine receptor, and that opens this ion channel that then causes a depolarization on the post-synaptic side and causes the action potential to be initiated on

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the muscle membrane. So that's of course the normal physiology.

The reason that the snail evolved this peptide is it's part of its paralytic strategy. And so what the peptide will target are these voltage-gated calcium channels. It's a channel blocker and very efficiently therefore knocks out functionally these voltage-gated calcium channels. And as a result, no neurotransmitter is released, and, therefore, the neuromuscular system is blocked, and the fish becomes paralyzed.

So if you inject this peptide into a fish, what you get is a paralyzed fish. So how do you make a drug out of that?

Well, it turns out that if in fact you look at what happens in the mammals instead of in fish, then of course neuromuscular transmission is pretty much the same. You have action potentials, and the same events occur. But there is one critical difference. And the difference is that, in mammals, the calcium channels are not the green calcium channels, but they're red and

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Prialt-resistance. And for those who are into calcium channels, the green calcium channels are Cav2.2 and, in mammals, the neuromuscular junction has Cav2.1 channels. And so in fact, this peptide is not paralytic when it's injected into any mammal.

However, we do have Cav2.2 channels, and it happens that they're in the pain circuitry. And so, of course, that is the basis for this becoming a drug because, now, as C fibers fire from the periphery in the synapse with this spinal cord neuron, if you block the release of glutamate at the synapse by adding this peptide, then what happens is that, even though you have a very powerful pain signal entering, because that signal does not cross the synapse, you therefore end up not perceiving the pain.

So what I think this tells us is, first of all, that the components of the nervous system are extraordinarily conserved. Whether the Cav2.2 channel is in fish or in mammals, this peptide recognizes it with very high affinity. It also can

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differentiate between this calcium channel and all other calcium channels that are present in any vertebrate system.

However, the reason this peptide is a drug is because, although the structure of these ion channels is conserved, their pattern of expression is not. And the fact that we have a lot of different expression patterns and different animals, gives us an opportunity, therefore, to take advantage of natural products that haven't been evolved over millions of years. And even though it's not targeted for the purposes that we would like, it turns out that because of this variable expression, very often you will find something that's very useful for the purposes that the biomedical community is interested in.

So what I'd like to do is to go back to the biology of the snails. And these snails, when they sniff a fish, extend their highly extensible proboscis. And this particular snail goes for the lateral line of the fish. And the moment it touches the skin of a fish, what happens is that,

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from that, it extrudes a disposable harpoon-like tooth, which also serves as a hypodermic needle.

So the venom flows through the proboscis, through the tooth that's extended, and it comes out at the other end. And as you can see, this is highly barbed, so this is both a harpoon and hypodermic needle. And so these snails got their opportunity 55 million years ago when, essentially, they evolved a drug delivery device that's the equivalent of disposable hypodermic needles.

(Laughter.)

DR. OLIVERA: So each snail has its own particular design. And what you see is how it's used. And so you can see the fish is tethered and venom is being injected. And in a very short time, the fish is immobilized. And now, the snail will completely engulf the fish. And in about an hour and a half, it will predigest the fish in this false mouth, called a rostrum, and it will regurgitate the scales and bones of the fish and the one harpoon that it used to inject its venom.

So that's how these particular snails make a

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living. So you can see that this is an efficient way for a snail to be able to catch a fish.

Now, if we want to understand the biochemistry and pharmacology of how they do this, we have to find out what the components are of the venom that are necessary for capturing a fish. Now, here is another snail, a related snail in the same clay.

What you're going to see is how fast this is. So they use a chemosensory mechanism to figure out where the fish is. And this is real time. And what you're going to see is just how quickly these snails are able to capture a fish.

So in this particular case, the snail doesn't even have to go above the substrate. And so this is a very, very efficient process.

So that's the fish-hunting cone snails. And fish-hunting cone snails have been the source of most of the work that we have done on cone snails so far. However, since the shells of these snails are pretty, there's a lot of interest from the collector community in classifying them. And so

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the molecular phylogeny of these snails is known. And in fact, it turns out that there are four different claves of fish-hunting species. And what I've shown you are species that belong to the F2 clave.

So let's look at one of the other branches of fish-hunting cone snails. And what you're going to see is that, in fact, there's a different strategy for catching fish. So if you drop a fish with one of these snails, instead of sticking out its proboscis, it opens its mouth. And, as you can see, it has a really huge mouth. You might say cavernous mouth.

Here's another species in that clave. And these guys always engulf the fish first before they sting it. Then they sting once it's in their mouth. And so it's a behavioral strategy that's completely different for how to catch a fish.

So what happens is that these snails crawl out of the reef at night. And what they do is, they approach a school of small fish that are hiding in reef crevices. And if they are lucky,

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they can bite the whole school and then pick them off one by one.

In order to have success at this, they have to be able to capture as much of the school as they can. And so they release a little bit of their venom. And as a result -- even before they inject the individual fish, they release a little bit of their venom. And the effect of the venom released is to make the fish act as if they are all spaced out, as if they are in an opium den.

(Laughter.)

DR. OLIVERA: Now, we called our groups of peptides that act together -- so if you look at all of the peptides that act together for a particular physiological purpose, we call that a cabal because cabals are secret societies out to overthrow existing authority. And so for this snail to capture a whole school, there are two cabals. First is the cabal necessary to quiet down the neuronal circuitry of the prey before it's engulfed. And then, after that, the venom is injected. The fish get all relaxed. And

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therefore, they can be eaten.

Those two cabaals, we call the one that causes sensory deadening the nirvana cabal because that essentially causes the fish to become hyperactive. And then when they inject the rest of their venom directly into the fish, that causes a complete paralysis and a relaxed fish. So it's this combination of nirvana cabal and motor cabal that these particular snails use.

So if in fact the nirvana cabal makes them hyperactive and puts the fish in a sedated, quiescent state, one might ask the question, does this have biomedical applications to quiet down overactive neuronal circuitry. And we have identified members of what we call the nirvana cabal, and here are a few of those peptides that are released by these particular snails.

It turns out that one can indeed use this for a variety of biomedical applications, and two of these have reached the human clinical trials. One of them, the sleeper peptide that Craig Clark originally characterized, it turns out, that's a

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subtype selective NMDA receptor antagonist, and that reached human clinical trials for epilepsy.

But the one more interesting to this audience is a peptide that we call contulakin-G, which really is a neurotensin homologue, except the snails have changed the N-terminus of the peptide and have glycosylated this threonine residue. But you can see that the C terminus is very similar to neurotensin.

This turns out to really be very, very effective and a very attractive compound in various animal models of pain. So this is potentially a new target for pain, the neurotensin receptor. And Allan Basbaum has actually looked at this peptide and found that it does have a lot of problems.

However, because it is a peptide, to get it -- it's also been injected into spinal cord injury patients in a clinical study, and efficacy was shown in that particular study. So I think it would be nice to be able to develop this one further.

So in addition to the approved peptide, a

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number of these conus peptides have now reached human clinical trials. And as you can see, they are not opioid like in their mechanism at all. But we are able to identify some new targets. And I'd like to tell you about the peptides of this class that target a certain type of nicotinic receptor.

So as targets, nicotinic receptors are complex because, of course, they are pentamers, and you can assemble these subunits in various ways. And we were really still not sure which of the different forms exist and which ones don't. So the molecular identity of the function of different nicotinic receptors is not completely known.

This is, I think, a general problem in neuroscience. To figure out the subunit composition of heteromeric ion-channel assembly just is quite hard. But it turns out that the snails know what's being assembled. And it turns out that conus venoms are a great source for peptides that are very highly specific for each of these nicotinic receptor subtypes. And the same Michael McIntosh who discovered Prialt is now a

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professor of psychiatry at the University of Utah. And he has defined a lot of the peptides that are very highly specific for each of these molecular isoforms.

So what is found is that there are peptides that target what's called the alpha 9, alpha 10 receptor and peptides that target the alpha 7 receptor. And these are the most closely receptors that you find, that are all alpha subunits, that are found in the human nervous system.

Curiously, these peptides do not come from a fish-hunting cone snail. Instead, they come from a clave of cone snails that eat worms. So it's a worm-hunting clave. And so these snails, what they really love to eat are fireworms. And so if you go scuba diving, you are told never to touch these things because they have really painful spicules, but that's the favorite prey of these particular cone snails.

That's the source of where we are able to find these alpha 9/alpha 10 nicotinic receptors, the all-alpha subtype. And the reason seems to be

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that, although we have this type of nicotinic receptor at a neuromuscular junction, invertebrates, nematodes, polychaetes, drosophila, they have a lot of subunits that are related to alpha 7, alpha 9, alpha 10.

I think C. elegans has 30 subunits of this type. And therefore, if you want to paralyze a marine worm, instead of targeting this type of receptor, they have involved a lot of peptides that target these types of receptors.

So again, this is the case where the human homologues of these receptors that are in the neuromuscular junctions of these invertebrates have very restricted distribution, but invertebrate systems are distributed all over the place. And so it really pays to screen venoms even though they're for a completely different purpose for things that might be specific for the type of ion-channel receptor that you want.

So what Michael has done is he's looked at what alpha 7 receptors do in various circuits and what alpha 9, alpha 10 receptors do. And it turns

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out that, if you inhibit alpha 9/alpha 10 receptors, this peptide is anti-nociceptive in nerve injury models of pain. And so what seems to happen is that if you have a nerve injury model of pain -- so we use the chronic constriction injury model. If you inject this peptide, then you reverse the pain that results from the nerve injury.

The basis of this is interesting because what the peptide seems to prevent is inflammation. And so when a nerve is injured, it's therefore attacked by macrophages, lymphocytes. And what you can see is that if you block this particular nicotinic receptor, then you count how many immune cells accumulated at the site of nerve injury. You can see that that's much decreased. And so you get less inflammation as a result of nerve injury by blocking the alpha 9/alpha 10 receptor.

Now, this just shows you how the pharmacology has to be really well-tolerated, because if you block the other closely related nicotinic receptor, the alpha 7 nicotinic receptor,

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what happens is it makes things worse. You get an increased attack of the immune system on the site of nerve injury. And the emerging story seems to be that if you activate the alpha 7 receptor, that's anti-inflammatory; but if you activate the alpha 9/alpha 10 receptor, that's pro-inflammatory. So when you inhibit that particular receptor, then you inhibit the nerve injury.

So what you can see is that by having very specific ligands, we can therefore have applications that are completely unexpected.

So what I'd like to do is take the last few minutes just to say that I think the future of venom peptides is that because these critters have evolved ways to recognize heteromeric combinations, that this is a class of targets that's very difficult to accurately target with small molecules, because differentiating between closely related heteromeric combinations is really a challenge.

So the worst ion channel family in that regard is potassium channels because there are

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70 genes, and the number of combinations that you can make for potassium channels is vast. And we really don't know which combinations actually exist and where those heteromeric combinations are.

So I'd like to take the last few minutes just to tell you about how we are approaching that. So what we do is, we take a region of the nervous system, in this case DRG neurons, where they are thought to be 25 to 30 different subclasses of neurons. And what we do is we culture them overnight, load them with Fura-2, and we can therefore measure intracellular calcium.

We can put about 200 cells in a well. And so here are a few cells. Now, if you add menthol, you can see that a few of the cells light up. If you have potassium chloride, then almost all of the cells light up. And so what we are doing is, we are using pharmacology to differentiate between the different cell types.

So here is an experiment where we're adding acetylcholine, ATP, histamine, menthol, and AITC. And what you can see is that these different cells

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respond differently. And so, clearly, these represent six different types of neurons. So if you monitor the cell population at this point, then you have low calcium in all of the cells. If you monitor calcium at this point, where you're adding menthol, you can see a few of the cells light up.

If you monitor calcium at this point, where you're adding potassium chloride, you can see almost all of the cells light up. So in this way, we are able to differentiate the different cell types of the DRG functionally.

Now, the other protocol we use is to add potassium chloride and to add a pharmacological agent before a pulse of potassium chloride. So if you add the tetrodotoxin, for example, of course, you decrease the influx of calcium. If you add a block of potassium channels, you increase the influx of calcium. And that's because, for calcium to enter when you have potassium chloride, you have to activate sodium channels, which then activate calcium channels. And then you terminate the excitation by potassium channels opening.

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So this is a very high-content assay because we are looking at all the sodium channels, all the calcium channels, and all of the potassium channels that are present in any of the DRG neurons and were simultaneously assaying all of those. And so we were using this as a way of discovering new compounds that are targeted to certain channels.

So we also make use of all of our other peptides that we know are specific for subtypes. And so we previously showed that some conus peptides are specific for KV1.2 and some for KV1.6. And so this just shows your field. We have genetically labeled some of the DRG cells. These are mice that David Ginty labeled. And now, we apply two of these peptides.

What you can see is that the majority of neurons are not affected by either peptide, so about 85 percent of the DRG neurons look like this. However, here's a class of neurons that are affected by the KV1.6-specific peptide, but not by the KV1.2 specific peptide. Here is a class of neurons that are affected by the KV1.2, not by the

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KV1.6.

The labeled neurons, it turns out, are not affected by the KV1.6-specific neurons, but are affected by the KV1.2-specific peptide, but the effects are very subtle, and we wouldn't have believed it if it weren't the fact that all of these neurons have a slight broadening when you add that particular peptide.

So you can see that, although this peptide was supposed to be specific for KV1.2, it actually had different effects on different neurons. And what I'd like to do is just to show you why that's the case.

So I don't have time to go into details, but we've looked in some detail at cold-sensitive neurons that are responsive to menthol, and they fall into two classes. The thermal sensors that are activated with mild cold temperatures and nociceptors, where you have to go to noxious cold temperatures.

Basically, what we find is that they have entirely different ion channels except for the

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menthol receptor. And now, if you apply these peptides, what you see is that the thermal sensors are not affected by these peptides at all, but the nociceptors are affected when you give them a menthol pulse. They respond much more to two peptides, one specific for KV1.2, one specific for KV1.1.

So originally, we interpreted this by saying nociceptors have KV1.1 and KV1.2. But our collaborator, Heinz Terlau, has shown that, in fact, the true target of the peptide that we thought was specific for KV1.2 is in fact this heteromeric combination because that peptide has a thousand-fold greater affinity for this heteromer than for the KV1.2 homomer.

So now, this is the forum of potassium channel that we believe is present in cold nociceptors. And so it is a problem, of course, of pain during chemotherapy, where what happens is during chemotherapy, you get a cold sensitivity. And I think we can explain that.

So the menthol receptor is present in both

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thermosensors and nociceptors, but the difference is that, in thermosensors, you have the menthol receptor, which then recruits calcium channels and activates the cell. But in nociceptors, you have the menthol receptor. And when it opens, the first thing that's recruited is this potassium channel, which is three KV1.2 and one KV 1.1 subunit. And of course, that repolarizes the cell.

So it's not until you go to very cold temperatures that you can overcome this potassium channel and begin to recruit calcium channels. And so this potassium channel therefore plays a role in how the temperature at which a cell is activated. And therefore, under chemotherapy conditions, it's the balance between the menthol receptor and this potassium channel that is dysregulated.

Therefore, if you want to cure that particular condition, this is the potassium channel that should be targeted. So I'm just making that point to show that, unfortunately, because of the nature of potassium channels, we don't really know the molecular composition of what we want to

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target. In this case, it turns out the potassium channel we want to target has three KV1.2 subunits and one KV 1.1 subunit, and we didn't know that before.

So I'd like to end by just saying that this has been a huge collaboration, both with colleagues in Utah and colleagues in the Philippines, Germany, California, and France. And here are the people who did the work at the University of Utah. Thank you very much for your attention.

(Applause.)

DR. BASBAUM: I have a practical question. I know that, in the case of Prialt, it can't be used orally, either because it won't get into the central nervous system or because of postural hypertension, peripheral actions. Is it possible, following along the lines of Joe's talk, where he's using virus to deliver a peptide that one assumes is going to be released by the neuron to act back on itself, have you ever tried or anyone ever tried to have these virally synthesized and then to get into neurons?

DR. OLIVERA: As far as I know, no one's actually tried it. I think it's been talked about, and people would like to do that. What people have done is they have expressed tethered forms of these toxins. And they have tried to introduce those into particular neurons by having the right viral vectors. And that's been tried, and it works, at least in some cases.

DR. BASBAUM: Is this in vitro or in vivo?

DR. OLIVERA: This is in vivo.

DR. MCMAHON: So one very naive question. Are the snails themselves resistant to their own toxins?

DR. OLIVERA: No. They are not. So if you put too many fish into an aquarium of fish-hunting cone snails, what happens is, you saturate their chemo receptors. And so, if they're hungry, they'll all come out, and they'll all have their proboscis out, and they'll sting anything that moves. And very often, they will sting each other. And individuals that get stung will die, not as quickly as a fish does, but clearly, they are not

resistant to their own venoms.

So as long as it's in their venom apparatus in their gut, they're okay, but if the venom is injected into the body cavity, they're sensitive.

So unlike snakes, where they have evolved nicotinic receptors that are intrinsically resistant to their own venoms, the snails are not resistant.

DR. BASBAUM: Roy?

DR. FREEMAN: So I've got a quick question. The primary receptor in the autonomic ganglia is also the nicotinic acetyl choline receptor. And I was wondering -- and this is a somewhat naive question -- whether those subunits, the alpha 7, alpha 9, alpha 10, were also present in that receptor.

DR. OLIVERA: I'm sorry. Which ganglion are you --

DR. FREEMAN: The autonomic ganglia.

DR. OLIVERA: So in sympathetic ganglia, you have alpha 3, beta 2 receptors and alpha 7 primarily. So as far as I know, there's no alpha

9, alpha 10, but there is alpha 7 and alpha 3, beta 2

DR. SVENDSEN: So do snails feel pain?

DR. OLIVERA: That's a good question. I suspect they do.

DR. SVENDSEN: How would you prove it? Because they have a different receptor system, and it might be -- somewhere along the line, that switch has been -- I mean, there's a lot of debate on whether fish and snails experience pain in the same way. But what you seem to have shown is a very different usage of the same --.

DR. OLIVERA: I think, among mollusks, probably people have done work in cephalopods. And I think that there is some work that shows that there's certainly somatosensation because they react by changing their chromophores. But I am not familiar with that work as far as I know.

DR. BASBAUM: No. The British government thinks that invertebrates don't feel pain. You need a license to do almost anything in Britain to an animal, but you don't need anything to do it to

an invertebrate. I think when that gene was cloned, the so-called painless gene in drosophila was a little bit annoying, just the notion that drosophila has a painless gene. So they have nociceptors. I mean, they will respond to injury, but to say they have pain, I think you do need a brain.

(Laughter.)

DR. BASBAUM: Another question I had -- I'm pretty sure you're familiar with Diane Lipscombe's work. So for the audience, the interesting observation that she made -- and I don't know where it's gone -- is that there is a splice variant of 2.2 in C fibers, selectively in the nociceptors, which raise the possibility that one could develop either a conotoxin or an antagonist that would take a 2.2 from the nociceptor and not get the side effect profile that would arise when the molecule gets to the brain, which can be pretty problematic.

Has that story gone anywhere? I really haven't seen much more.

DR. OLIVERA: At least not

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pharmacologically. I haven't seen anybody try to develop a specific pharmacological agent that distinguishes between spliced variants. But it's a good idea.

DR. BASBAUM: I think we've had a long morning. Why don't we have lunch? And then we'll get together after lunch. Thank you all for an entertaining morning.

We're doing that now? What? Are we cancelling lunch? I'm sorry. I was concerned about the time. Tell you what. We'll try. We'll have an overall discussion for a half-hour and then -- I'm just jet-lagged. I didn't sleep well last night.

(Pause.)

Q&A and Panel Discussion

DR. PORRECA: So I think it's open questions for the panel, and Mac has the first question.

DR. MCMAHON: I was told to ask questions. So I thought I would ask the same question that I asked Yves yesterday. And that is, you all presented different technologies, different

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approaches, that seemed to be a fantastic promise for the future, but what do you know about them that makes it difficult? Or what's the downside of each of these approaches?

DR. GLORIOSO: I can start with that one. The hardest problem about doing gene therapy for pain is delivery to the right cells. And we have debated this a lot. And the reason I picked on post-herpetic neuralgia is because at least we can identify a dermatome where pain is arising and might have a good shot. But there are many other types of pain where it's maybe a lot more problematic to deliver the vector.

DR. SVENDSEN: I guess, just to go on, with the stem cell delivery, we haven't done it in pain models yet, so we don't know what the problems will be, but I imagine that you have different growth factors one could choose and different peptides. And it's going to be a case of choosing the right one. And GDNF, based on your work and many other papers, is the best bet.

It's not a very clean pharmacological agent.

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But my experience is, the dirtier the drug, the more likelier it is to work. And I think when you don't know the origins of something, it's kind of a shot in the dark, but at least try it. But I think the delivery seems pretty straightforward for us in that the cells release where they are.

So I think we can get it delivered. Just will it work? That's the main problem.

DR. FREEMAN: So picking up on Steve's comment -- or question -- and Joe's comment about delivery to the right cells, early on, in fact, I think there's still some ongoing studies in neurodegenerative disease, intrathecal therapy, and there was very early on intra-arterial injection therapy, where using stem cells was proposed.

I wanted to hear some comments. Obviously, the ease of delivery is much greater, and there will certainly be much more uptake if the microinjections were not involved. So I want to hear both from Allan and Clive what your view is on those modes of delivery.

DR. BASBAUM: Many years ago, Jackie

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Sagen -- actually, she even had a company. I don't remember the name of it. It didn't last too long. But the approach there was intrathecal delivery. And there were some patients, and what they were using was originally bovine adrenal cells because they released enkephalin. Adrenal cells make buckets of enkephalins and norepi [ph], the two of which independently, at the level of the spinal cord, can be anti-nociceptive analgesic through an alpha 2 site and through an opiate receptor.

They implanted these cells intrathecally initially in animals and then in humans. There were a few humans that were trialed. It was in France. It was Yves Lasorte, the neurosurgeon there who did it.

The problem there is that that's a pump. There's no question. That's all it is, is a pump. And then, do the cells sit down? They don't penetrate. What happens to the molecules? And it just didn't go anywhere.

So I'm not sure that that's any different from just putting a pump in and having a catheter

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that will put out the compound for you. I do think there's a big difference between putting it in intraparenchymally.

DR. RICE: Just to comment, if my memory serves me correct, the other reason that died was because it was bovine tissue. It was exactly the same time as the bovine spongiform encephalopathy crisis. And I think it was a safety concern that essentially killed it rather than an efficacy issue, although efficacy may have been a problem. But it was bad timing.

DR. BASBAUM: Okay.

DR. PORRECA: Andrew, could you continue? So in what circumstances would an ethics committee approve this kind of a therapy, and what types of patients might be considered?

DR. RICE: Tough question. I mean, Jacquelyn Sagen's idea was killed for a very specific reason at the time, that may or may not have been a problem. It's something we discussed in the coffee break. Because of the potential downside of this, I think an ethics committee would

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have difficulties -- very much my personal view; you asked me the question -- with a non-fatal symptomatic condition, although of course they have given approval for retinitis pigmentosa, for example.

But that's why I suspect that conditions like ALS, which I have very personal experience of, would be much more likely. And it's also associated with a rapid death. But that's a very personal view, and there's people who know more about ethics in the room than me. But I think it's going to be difficult to justify paying for such approaches.

DR. SVENDSEN: I kind of agree. It's a cost/risk benefit. But I don't know. I'm not familiar with the pain syndromes enough, but I imagine there are certain pain syndromes where there's nothing. And you know, the chronic pain, the risk of putting some cells in the spinal cord -- the good thing with ALS trial, as we've shown now and Boulis has shown, we can put cells in the cord without doing too much damage -- as we get

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better with the surgery and less invasive put them in.

Just going back to the last question, if we put the cells in the CSF, if we put them in the blood, they don't penetrate into the core. In our experience, mesenchymal cells don't get into the brain. Neural cells don't get in. And I think UCSF would agree, you have to do them intraparenchymally. We'd love in stroke to be able to get in through the blood. And we've tried, and tried, and tried, but we have to go intraparenchymally to get the cells in and integrate it.

So what pain syndromes would you think -- maybe the FDA's in the back -- would be severe enough?

DR. BASBAUM: I really disagree with Andrew.

DR. RICE: You asked the question.

DR. BASBAUM: No. You suggested you would only want a terminal condition, and this is a true story. On the first grant that we submitted on this proposal, it didn't get funded. It did

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eventually get funded, but one of the reviewers wrote, "Why would you ever want a transplant therapy for a non-life-threatening condition like chronic pain?"

To me, that's an unbelievable misunderstanding. And you no better than most, there are some patients who will actually commit suicide when you can't treat them. If the mantra in the field is that the best there is in some of the neuropathic pain conditions is 30 percent efficacy and 30 percent of patients, then what do you tell the rest of the patients?

So the real issue is risk/benefit. And that's the question, but that's no different from any drug. If you have a spinal cord injury case, where 70 percent of those individuals have intractable pains -- that is one of the things that makes their life so miserable -- is that potentially a therapy where there's already damage to the spinal cord?

I think the real question is how much damage is produced by the intraparenchymal therapy. And I

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don't know the answer to that, but surgeons have been clearly going in, and putting probes in, and putting stimulators, intrathecal pumps, injecting alcohol into trigeminal ganglion.

So I think one can manipulate the nervous system quite considerably. And it's a matter of time. We'll find out when it's tried.

DR. PORRECA: Just to clarify, I mean, the 30 percent efficacy number that you mentioned, I think you have to compare it with intrathecal delivery by pump of drugs that we have available. And I think that that number is considerably higher.

DR. BASBAUM: I agree.

DR. MCMAHON: [Inaudible – off mic] -- provocative. Surgeons do lots of things that are not necessarily good.

(Laughter.)

DR. MCMAHON: There's a big argument about surgical fusion for low back pain. I mean, is that a good thing or a bad thing? I mean, someone does it because the people are desperate doesn't mean

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it's a good idea.

Now, when the stem cell therapy started, one of the questions asked was -- so maybe I can ask you -- how sick would you have to be to have this very invasive procedure? I mean, I would not have it for a bit of sciatica. I just wouldn't.

DR. RICE: I can see your point of view exactly, and I don't -- I was putting in an extreme point, but I can see the corollary, for example, with heart transplantation.

In the beginning, before we knew it worked, we only took the very, very sickest patients. Now, it's not a routine procedure, but it's done on people who are really quite healthy because they're better potential recipients for a transplant.

So I guess the point I was making is that we probably need to look for the dangers and the issues in the fatal conditions before we go on to symptomatic conditions. That's the point I was making.

DR. BASBAUM: Be provocative, Frank.

DR. PORRECA: I could be. I could ask you

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about intrathecal baclofen and its efficacy in pain.

DR. BASBAUM: That actually is one of the more disappointing things, I have to admit. So Frank is asking the question. GABA-B agonists -- there are GABA-B receptors on primary afferents, on post-synaptic neurons. Baclofen, which is a GABA-B agonist, particularly effective in animal models, in a variety of animal pain models that have been intrathecally.

It is not effective for pain in humans. It's effective for spasticity and maybe the pain secondary to the spasticity, so it's used in MS patients, for example.

That's a puzzle that I don't understand. That's one of the examples of poor translation. Now, is it also possible that when you give a GABA agonist, that it's targeting both GABA-A and B and, together, the two might be effective?

Again, it's empirical. We know that GABA-A agonists can help, and some of the benzos probably enhance the effect of some of the existing

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compounds. You have a side-effect therapeutic window if it starts to get to motor neurons.

I think the GABA-B story is one of the puzzles. As far as I know, the only pain condition in which it ever really has worked is trigeminal neuralgia. The neurologists can concur it's not used anymore because there's better drugs.

But I would then argue, because I know where you're going, Frank, if I were to transplant, then, trigeminal neuralgia, it may be actually a terrific first place to go.

Am I right about the baclofen?

DR. PORRECA: Yes. Misha?

DR. BACKONJA: Just to kind of inject a little bit of clinical perspective as one of the clinicians here, to argue for some conditions, that they're really painful -- and as Allan pointed, some patients really got to the point of suicide, where pain is so intractable. Again, we don't have statistics, but if you talk to any clinicians who have a large practice, that's one of the things that does happen, unfortunately.

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So as already stated, probably spinal cord injury or post-central pain syndromes, they're very level [indiscernible] one would consider. And especially, if there's any evidence that would be of benefit to some neural recovery, their sensory motor, or any, would be a benefit.

So maybe it's one of those things, as we talked about yesterday. We should widen our scopes, what we're measuring, if you're indeed looking for motor recovery. Also Clive and his team is literally working on pain as well. He's looking for adverse effects, but also potential recovery. So it's an appropriate strategy and something to be considered.

But regarding the efficacy of intrathecal therapies, our feeling in managing so many pumps, they were nearly not as effective. I mean, there's really no good trials to compare, any comparator studies. But one of the big issues in intrathecal delivery of drugs is, there are a limited number of things that are proved, but in clinical practice, a number of things are utilized. And unfortunately,

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we end up doing in intrathecal therapy what they do in systemic therapy, which is frequently combination therapy.

Again, something that's evolved as a clinical practice, we're thinking that maybe one system is addressed, but there are other systems that are not. By combining therapies, you will look at some efficacy.

The sad story in all of this is, there's really no good prospective data. So one hope is that with the registries that are now being implemented, led by the Stanford effort and Huro [ph] is a part of PROMISE effort, that we will learn from real-life experience if it works or doesn't. But there's really so few data points regarding the comparison.

DR. PORRECA: Yes. But I think that our understanding is emerging with the intrathecal therapies. And I know that there is a lot of observation at the moment, and different clinicians use different drugs and/or cocktails of drugs to produce or try to titrate the effects for

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individual patients. I think that understanding will increase over time.

Also, kind of an interesting emerging story, even in patients that are not responding to systemic delivery of opioids, that weaning them off the opioids and then beginning an intrathecal pump seems to potentially be effective.

So a lot of questions that I think we don't understand, but there may be still more to learn with a mechanism that potentially could be effective in some patients.

DR. BACKONJA: Another unfortunate observation against intrathecal therapy is that, frequently, there is development of tolerance, which is really a big problem.

DR. PORRECA: Well, sometimes.

DR. BACKONJA: A lot of times.

DR. PORRECA: I don't know.

DR. BACKONJA: Clinical experience.

DR. PORRECA: Yes.

DR. BASBAUM: I wonder if I could ask Toto a question. So Prialt has been around for quite a

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while now. And you showed that some of the other toxins are in trials. I'm actually kind of curious, because there are so many -- I mean, I was familiar with the contulakin, as you mentioned. But what is slowing down the development? Prialt had an interesting history, as you know. I mean, it can be incredibly effective. It had a weird side effect profile, and I think it scared a lot of people, but it can be remarkably effective.

As you said, there's so many potential targets. Is there some explanation for the relatively slow clinical development?

DR. OLIVERA: So I think it's the delivery problem again, intrathecal delivery. There's no financing at the moment to push a drug for a market of only intrathecal patients that have to. So there are a number of strategies.

DR. BASBAUM: Chas still here?

DR. OLIVERA: Yes. There are a number of strategies to try to make these more systemically delivered.

DR. BASBAUM: Can you talk about that?

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DR. OLIVERA: Well, one of them is to circularize the peptide. And in a number of cases, if you have no ends, that's much more resistant to proteases, and so you can put massive amounts into the circulation. And you can also modify the peptide to get more of it across the blood-brain barrier. And so people are trying that.

DR. PORRECA: I don't think that Prialt is universally effective. I think there are some patients that respond, but there are plenty that don't, which is kind of interesting from a mechanistic point of view as well.

Yes?

DR. GLORIOSO: I wanted to ask Clive a question, which is that I'm a big fan of iPS cells as well, and they're great as model systems for understanding disease. So I was wondering, in the case of deriving iPS from ALS patients and transdifferentiating back into neurons, what are the differences between those neurons, if any, and the neurons that are normal?

DR. SVENDSEN: That's another whole talk.

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But I think that the lessons we learned from spinal muscular atrophy, like I showed, when you reprogram skin cells from a patient there, where the motor neurons die, and then you push them forward again to be motor neurons, after six weeks they just start dying, just like they did in the kid.

When we did that with ALS patients, which is an adult onset disorder, we reprogrammed them. We then pushed them to be motor neurons. The motor neurons looked pretty happy. And in fact, they lasted six weeks. They lasted 10 weeks. They didn't undergo that death -- with the patient.

Now, maybe we have to wait 40 years or 60 years, and the graduate students weren't that keen. So maybe we have to accelerate, perhaps, the differentiation or aid the cells somehow, artificially, to bring out a phenotype. But we did see some more interesting phenotypes, which were electrophysiological changes in the cells, more firing. So there were, actually, interestingly, some phenotypic changes in the cells.

DR. GLORIOSO: So if you do the obvious,

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like look at transcripts, and profiles, and things like that, what do you see with that?

DR. SVENDSEN: Beautiful transcript. I have another whole talk on big data. And I think one of the things that's coming out is they do have a very unique transcription profile. We did RNA seq, and you can tell the difference between HD, SMA, Parkinson's, and ALS just on the transcript done. And it segregates beautifully into those with non-bias -- so you can do it on the transcript.

DR. GLORIOSO: So you're desperate for models, so have you tried making transgenic animals with the cells? In other words, use them as stem cell for generating animals.

DR. SVENDSEN: That's a little bit further on than our IRB would probably go. I'm not sure what you mean.

DR. GLORIOSO: Other people have taken iPS cells and put them into developing embryos, and gotten mosaic-type animals.

DR. SVENDSEN: We're constricted by our SCRO committees as to what we can do with the human iPS.

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You're not allowed to put them back into developing monkey embryos, for instance, or even rat embryos by the SCROs.

DR. GLORIOSO: I see. That's right. You have human cells, so you can't do it. I'm sorry.

DR. SVENDSEN: Yes. So they get very nervous that you're going to --

DR. GLORIOSO: Yes, of course.

DR. SVENDSEN: -- make a mini-human. But what we are doing is making organoids. And we can almost make now a spinal cord organoid where the cells self-aggregate and start making something that looks a bit like a spinal cord, so we're getting closer to that technology.

But I just wanted to go back to the last discussion briefly and the consensus. And something that would be very useful to me, and I'm sure to Allan and Joe is -- because we're all on the edge, it seems like, of clinical -- all four of us, clinical trials.

Me, as an outsider to this field, I was talking earlier on with Steve. If we had a list of

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the appropriate diseases, we could then pick the appropriate animal models that would gel with the diseases, the types of pain that the clinicians say this is what we need, and have some kind of consortium effort -- maybe that the FDA would be interested in funding or the NIH -- to, in a small way, just get the experts together and put a translational team in.

We talk about this at meetings, but then you go away and you start to get busy. But it would be nice to have a consensus because all of these therapies sound very exciting, but I know the problem. We had $20 million from CERN, but it's still a struggle. I had to reshape my whole lab to become a mini-company, essentially. It doesn't happen easily. And if we're all trying to get our neuron papers, it's almost impossible.

So why not have FDA and Pain and try to get a consortium? And we're trying that with some other diseases. It seems to be working. And you need the clinicians to guide us, because we could get the best animal model that's completely

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irrelevant for pain in humans. I think I've heard that today.

So is that something that could come out of a meeting like this? I don't know.

DR. BASBAUM: Yes.

(Laughter.)

DR. PORRECA: Other questions or comments?

DR. BASBAUM: I think people are hungry. Why don't we take a break, have some lunch, and we'll continue after? Thanks, everybody.

(Whereupon, at 12:19 p.m., a luncheon recess was taken.)

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A F T E R N O O N S E S S I O N

(1:23 p.m.)

DR. TURK: For those that don't know me, my name is Dennis Turk, and I'm one of -- the small font. Those of you who were here yesterday, you saw there was a big font who organized the meeting, and then the small font. I'm one of the small-font people, with Bob Dworkin.

I just wanted to add my thanks to all of the speakers for their presentations. I think this has been one of the most stimulating conversations, discussions. And what's really been gratifying is that, number one, hearing that from some of the other people in the audience, but number two, just looking in and watching the interactions that are going on among the people who are here.

I think the most gratifying thing is really seeing how much stimulation, how much excitement, and enthusiasm. And even for those of us that some of these topics may not be exactly what we normally work in, it's an opportunity just so we get some overview, and perspective, and some understanding.

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So thanks to all of the speakers. I think we've had a tremendous day and a half, and I want to thank you.

Some people may not have been here, and I want to remind them that we want to emphasize that these sessions have been videotaped, and there will be a transcript put on the ACTTION website. And I know some people have said I'd like to have students see this or I'd like to have colleagues see this. And I think it's probably three or four weeks, it'll be up. It takes us about that long to get there.

So it will be available to you. And we hope, Bob, I, Allan, and Frank, that in fact this will go beyond just the two days of the meeting, but we'll actually be stimulating you.

So thank you all very much for being here. Thank you for staying. And thank you for your enthusiasm that we've heard so far.

In one of the previous scientific programs that ACTTION had conducted, there was a lot of discussion about the failures and the difficulties

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of doing some of the trials, and the inability to bring new drugs forward, and that the pharmaceutical industry was actually losing enthusiasm, losing interest, backing away from the neuroscience area, the pain area. We heard Chas Bountra yesterday tell us a little bit, reinforce that particular concept.

One of the things that we did as we organized this meeting, we were thinking, is there a way to try to get away from some of that gloom and doom? Are there some successes that would be useful for people to hear about, what the procedures, what the studies are, and what are some development areas. So it's not just that there are areas where we've had failures, but there are in fact some successes.

So what we were hoping to do this afternoon, at least the first session, is to cover some areas that have been relatively successful attempts from both the pre-clinical and moving into the clinical area, to start looking at some areas that may be promising, not that we guarantee or anybody can

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guarantee anything is going to be the perfect solution to the dilemma, but at least to see that there are in fact some promising directions and things that are happening.

So I'm going to be co-chairing this with Bob Dworkin. The first speaker is going to be Andrew Rice. I want to thank Andrew especially, one of the people who came all the way from U.K., a long distance, and we appreciate that. However, I have a hunch, coming from Seattle, where I live, it may have taken me about as long as it took you to get here.

Andrew is going to be speaking about some pre-clinical and clinical studies that he's been involved with on angiotensin 2 type 2 receptor blockers to give us a perspective of how that process evolved, not just the outcome, I guess I'm hoping, but how it developed and how it moved along, so that maybe it will give us some guidance as we think about studies going forward.

So Andrew from Imperial College London, you're on.

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Presentation – Andrew Rice

DR. RICE: Thank you, Dennis, and thank you for the invitation to talk about the angiotensin 2 type 2 receptor blocker program that I've been involved with Spinifex in, and really just to point out that perhaps I'm just a spokesman for this. There are several people in this room who are also involved in that program. And their names will become apparent as I run through the presentation.

First of all, my conflicts. I've been involved with Spinifex since 2005 as a paid consultant and advisory board member, and I've been engaged in both pre-clinical and the clinical trial development activities. I received research funds from Spinifex for my lab at Imperial for two sets of pre-clinical studies in 2005 and 2007. And I own share options in the company.

So just a little briefing in terms of the renin-angiotensin system, because this is really the first thing that would spring to mind when you're thinking of potential neuropathic pain targets.

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For those of you who know me, you might know that I enjoy history. And there's a lovely piece of history in that the discoverer of the system was a hypertension researcher, Stan Peart, who was my first teacher at medical school some 40 years ago, so it's a nice return to history for me.

Angiotensin 2 is the active substance in the system. It is formed from two precursors. And until relatively recently, it was known that the major pharmacological site of action of angiotensin 2 was the AT1 receptor, which is involved in blood pressure regulation and various other cardiovascular functions.

Interestingly, some of the genes associated with this have fairly recently been elucidated by colleagues at UCL as being involved in high-altitude syndrome and potentially survival in intensive care units and endurance athletics.

As I am sure you're aware, there have been two broad strategies in terms of cardiovascular disease involving the system, angiotensin-converting-enzyme inhibitors and angiotensin 1

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receptor antagonists for hypertension.
82 has been investigated in several spheres. Much less is known about the 82 receptor, particularly its intracellular signaling mechanisms, and really haven't been thought of as a drug target. And I think our trial is the first 82-receptor antagonist to successfully have gone through a phase 2 clinical trial.
The first important point to make out is that 82 is not involved in the control of blood pressure, although of course we did look carefully for cardiovascular adverse effects.
I'm going to talk about a number of compounds, which were used in the pre-clinical development. The most important one is EMA401, which is the sodium salt. And that was the one which we used in the clinical trial, and it has selectivity for 82 over 81 receptors. Some of these early ones are also referred to by old Parke-Davis numbers.
So bearing in mind the nature of this meeting, I did look at some of our G microarray

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data, where we looked at various animal models, and we can see that the gene for angiotensin 82 receptors is decreased in the rat dorsal root ganglion in rats that have been subject to spinal nerve transection. But it remains at levels in a model of HIV neuropathy. But we also know that it networks with other pain genes.
There are other angiotensin signals, sometimes conflicting, hinting at involvement in pain. For example, we know that consumption of ACE inhibitors is a risk factor for complex regional pain syndrome, type 1.
There's a paper in Cell at the moment which shows that a Baruli ulcer, which is a microbacterial infection associated with painless ulcers, isn't like another microbacteria, leprosy. The painlessness in that is not due to destruction of sensory nerve fibers. It's shown to be an angiotensin signaling mechanism. And there are various other hints that it may be involved in pain and nociception in some way.
Studies in Smith's lab have shown that

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estrogen-evoked neurite sprouting in small medium cultured dorsal root ganglion cells is eliminated by AC2 receptor blockades, so it appears to be active at the DRG level. And the 82 receptor, but importantly not the AT1 receptors, is localized to small- and medium-sized neurons in both human -- and in Praveen Anand's lab, it's been shown in the human cells and also in rat DRG, and trigeminal ganglia.
I think quite an important point is that the expression of angiotensin to receptor doesn't appear to change in the DRGs of rat nerve injury models, but expression of the ligand angiotensin 2 does increase.
So Praveen Anand is involved in this Spinifex development program and has done a lot of work with cultured rat and, more importantly, human DRG cells, including those taken from patients with brachial plexus avulsion. And it's shown that the EMA401 82 receptor antagonist that we used in the clinical trials -- we used the sodium salt in the clinical trials -- inhibits capsaicin-induced

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calcium influx, and, conversely, angiotensin 2 itself enhances that effect. And 81 receptor and blocker, losartan, had no such effect. Rather like as has been shown in Smith's lab, angiotensin 2 enhances neurite outgrowth and EMA401 is a type that inhibits it.
We also know that CFA induces proliferation of dermal and epidermal sensory nerve fibers in skin and 82 receptor antagonists prevent that. So there appears to be some involvement of neurite sprouting under nociceptive conditions or pain conditions.
I'll come on to Maree Smith's role at the end, but probably one of the most important studies was the inventor of this technology, Maree Smith at the University of Queensland in Brisbane. And this is one of her early studies, although we desisted in publishing quite a lot of this pre-clinical work until we had the confidence of a clinical trial.
But she showed that a number of angiotensin 2 receptor antagonists do act as analgesics in the CCI model of peripheral nerve

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injury and has shown this for three of the development compounds that we've used, with the last one being similar to the one that is used in the clinical trial I'm going to talk about.

There's a lot of talk about replication of pre-clinical data. And we took a decision sometime ago. These were experiments performed, at least in my lab, in around 2007. But we needed to replicate the work in my lab and Maree Smith's lab with regard to the activity of these compounds in neuropathic pain models, and we just also took a decision to publish these together.

Now, certainly, my view and, I think, most of you who would work in this area would agree that exact replication of a protocol between two distinct laboratories is really, really difficult, if not impossible. There are so many confounds, the supplier of the animals, the exact strain of the animals.

So what we did, which was different, was to ask both labs to replicate the question. In other words, are 82 receptor antagonists or the same 82

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receptor antagonists effective in animal models of antiretroviral toxic polyneuropathy? And there were variations between the study, but the important thing is, they came to the same result.

On this slide, you can see the results from Maree Smith's, and there's a slight difference in the dosing, the effective dosing, but they're in the same ballpark. And we were both able to show that the highest dose is examined. The efficacy in this model of toxic neuropathy was roughly equivalent to that of gabapentin. And we were able to show dose-response function.

But I think the important point is that it was replicated in two independent labs, about as far away geographically as you can get, London and Brisbane.

I am very keen that we declare various factors concerned with how we did our studies. And these are pre- the ARRIVE guidelines. They were done again in 2007, but we were able to report inclusion and exclusion criteria, randomization, blinding, et cetera. We didn't do a sample size

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calculation. That would be different today. And concealed allocation, again, we didn't do, but that would be different if I was to do the study today.

We also commissioned some studies in Aberdeen from Cameron and Cotter in the streptozotocin-induced model of diabetic neuropathy. And these were fascinating to me. Firstly, they were able to show that, similar to gabapentin, an 82 receptor antagonist here had similar efficacy in terms of mechanical and thermal hypersensitivity in this model, and this was sustained over a period of time.

More interestingly -- and it's something we haven't actually pursued in the clinical program yet; we're still discussing it -- the nerve conduction velocity of both motor and sensory nerves was restored with the 82 receptor antagonists, but not with gabapentin, which may have some reflections in terms of the mechanisms that I was talking about. And they used the sodium salt of EMA401, which is the same compound we used in the clinical trials.

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Maree Smith, in a recent publication, has also showed that in 82 receptor knock-out mice, the effect is largely abolished and has suggested the inhibition of MAP kinase is maybe one of the intracellular signaling routes involved in that, in dorsal root ganglion cells.

I won't run through the pre-clinical toxicology. We obviously looked quite hard for cardiovascular signals, except to say that there really wasn't anything that has unduly alarmed us. One quite interesting feature that we discussed a lot at the time was in the phase 1 human studies. We saw episodes of non-sustained ventricular tachycardia both in the placebo-treated people and in the drug-treated people.

I think there's an increasing awareness, with that kind of monitoring, long-term ECG monitoring, you are likely to see these adverse events that, in our case, don't appear actually to be related to the drug, although we did go and look if there was a potential mechanism for it, and there appears to be no association with the drug

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effect. It was a coincidental finding.
So that was a brief summary of the pre-clinical program. There were some differences to other pre-clinical programs, but nothing actually stands out as really obvious to any other pre-clinical program that I have been involved in.
So we went on to a phase 2 clinical trial in post-herpetic neuralgia. It's a rather typical design. We had a four-week treatment period of EMA401, and it shows post-herpetic neuralgia as the clinical trial model, I think, for two reasons. It was something we discussed an awful lot.
The first is that, as Bob Dworkin and his colleagues have shown, you're most likely to see an effect, which is of course what you want to do in a phase 2 trial, with post-herpetic neuralgia compared to other neuropathic pain models that are used in clinical trials.
Secondly, to me, if you're looking at excitatory or blocking excitation in hyper-excitable DRG cells or sensory neurons, then having a clinical trial model where there is both

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patients with sensory gain and sensory loss phenomena makes sense, whereas largely in, say, a diabetic neuropathy model, you may just see sensory loss phenomena.
So inclusion criteria were pain for PHN of more than six months' duration. One thing that we did do that I've not seen done before is that the patients, the investigator, and the sites were all masks for the inclusion criteria. And we used an algorithm that is still confidential, that excludes patients on the basis of the numerical rating scale for pain variability and excessively high scores, and excessively low scores, and whether they completed their diaries.
Now, we haven't published that trial because it would lose its masking effect for future trials, but we are prepared to share it with people who are proposing to use it in other trials, if you contact us. In terms of con-meds, we allowed a maximum of one drug at stable dose.
The study protocol is now freely available, full study protocol, at the Spinifex website. And

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it was registered with the Australian and New Zealand clinical trials registry. Spinifex is originally an Australian company.
We recruited between August 2011 and May 2012 at an estimated sample size of 77 per group in 6 countries and 29 centers. The centers were in eastern Europe and South Africa. We randomized 183 patients, assigned 92 to EMA401 and 91 to placebo. And of those, very few patients dropped out. 86 completed the EMA401 arm and 83 patients completed the placebo arm.
I'll come on to the adverse events profile, but just to say at this stage, there were very few patients who dropped out because of adverse events, but we didn't get a serious-adverse-events signal.
The demographics are very characteristic for a PHN trial. The average age of the population was around 62, 63. There was a slight female preponderance, as you'd expect in a population of that age. And they have had PHN for a duration of, on average, about two years with a baseline pain intensity of around 6, 6 and a half.

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Because of the countries that we recruited in, the number of con-meds were not similar to what you'd expect in trials that were perhaps done in Europe or North America. But a small number of patients were taking either pregabalin or gabapentin. They were the most common con-meds, or some certain anticonvulsant drugs. Capsaicin, 8 percent, isn't available in those countries yet.
The primary efficacy pain endpoint is pain intensity comparison between baseline and the final dose, end week. And we did both BOCF and LOCF imputations. There was actually no difference in the outcome, but the primary was on LOCF.
We saw a gradual increase in efficacy across the trial in comparison to placebo. So by weeks 3 and 4, there's a clear separation of EMA401 from placebo.
I should emphasize that there was no dose-titration phase, and we have had some discussions as to why the efficacy takes a little bit of time to appear. And this is clearly something we'd want to investigate in future clinical trials.

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We did a number of secondary and comparative efficacy studies and looked at both the percentage of patients to achieve 30 percent pain relief and 50 percent pain relief. And they come out with, if you like NNTs, at about 4 and a half for 30 percent and about 6.7 for 50 percent.

Now, there's a strange thing that happens when you're flying across the Atlantic, in that we seem to favor the 50 percent in Europe and 30 percent in U.S.A. They've both been justified. But we also took an idea that John Farrar published a few years ago. Actually taking these pretty arbitrary 30 percent and 50 percent fixed measures of responder rate are somewhat illogical, and we produced this cumulative estimation of responder rate, which I think gives a much better, certainly visual depiction of the efficacy against placebo. And certainly, I think that's a useful way of showing that information.

Just before we published the trial, Andrew Moore, fairly typically, published a fairly innovative and thought-provoking way of looking at

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success for neuropathic pain -- neuropathic pain efficacy in the BMJ. I won't go into it in detail because it takes time to explain, but basically, it's a computation of success and failure rates of drugs based on the difference between the maximum effect that you can achieve against the active drug and the difference between active drug and placebo.

But basically, what this computation tells us is that the efficacy of the drug that we're looking at, the EMA401, is very similar to that seen by pregabalin, and topical capsaicin, and gabapentin, although quite an important point to make is that we were looking at it in the context of a four-week treatment period, whereas the duration of the data for the other drugs is in 12 week, because they're much further ahead in development.

We had a number of other pain efficacy measures, and we're very encouraged to see that, generally, they all move in the same direction. So on the new version of the short form McGill, all the parameters separated between placebo and EMA401

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with the exception of intermittent pain, which is probably what you'd expect for post-herpetic neuralgia, and the scores on the brief pain inventory measures did, as did the patient impression of change. We used the Insomnia Severity Index, and that just failed to reach statistical difference between placebo and EMA401.

So in general, all the secondary outcome measures for efficacy measures for pain moved in the same direction as the primary.

We didn't see this for depression, anxiety, and positive outlook or pain catastrophizing, but I think you really wouldn't expect to see that in a four-week study. And also, certainly my personal view is that these are probably more useful as stratification measures than outcome measures.

I'm not sure how often they actually change, even in longer pain trials. And I think that would be quite an interesting discussion to have around the group, as to where we ought to be placing these measures in clinical trial design.

There was really nothing to separate EMA401

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from placebo in terms of the adverse events with the exception of a slightly increased number of people that treated with the randomized EMA401 that had headache. And there were a couple of L3 episodes of allergic dermatitis in the EMA401 group and one in the placebo group.

I think I may have missed the slide that showed the number of centers. We did a per-country analysis that was preplanned and saw no difference in the efficacy between countries. Because two centers recruited a large number of patients, there is a center effect, but we've looked very closely into that, and we don't think it compromised the results at all.

So we've now secured venture capital funding for the rest of the phase 2 and getting into phase 3 plans. And we are currently discussing this. These are not yet set in stone, but we've put them in a press release to date. We are planning to do a phase 2B multidose placebo-controlled trial in PHN with a 12-week treatment period.

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We don't think we have hit the full efficacy dose yet because we really weren't seeing adverse effects in this trial, so we think we can push the dose somewhat higher. And we're also discussing other clinical proof-of-concept studies at phase 2 level in other neuropathic pain conditions. And I'm sure we'd love some feedback from the meeting as to the relative importance or usefulness of different trial models, but we are certainly looking at diabetic neuropathy, and we're also considering non-neuropathic pain conditions such as osteoarthritis, although the pre-clinical rationale is not that strong.

One study I have left out is that Maree Smith has also recently published efficacy and pre-clinical models of cancer-induced bone pain.

So that's really a brief summary of the EMA401 story to date. We're at phase 2. We've got a long way to go. You will have seen from the authors the people who have been involved, but I would like to highlight two particular people who deserve special mention in regards to this program.

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First is Maree Smith, who is basically at the University of Queensland. She is essentially the inventor, came up with the original hypothesis, and made contributions to some of the pre-clinical data. And Tom McCarthy, who is here, has been the CEO of Spinifex. We worked it out. It was eight years now, Tom. It seems shorter. And Tom has done a sterling job in leading what is a very small company so far, not only through the scientific aspects, but dealing with the venture capital funding for the company.

So thank you for your attention, and I'm happy to answer any questions.

(Applause.)

DR. TURK: Questions for Andrew?

DR. MCMAHON: So thanks. I think Chas is back again. Now, after his pessimism the whole of yesterday, it's good to see some positive news in the pain field.

But a few things, really. One, do the patients know whether they're on placebo or compound? I mean, is there any unblinding from

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their perspective, if they guess?

DR. RICE: We didn't specifically look for that, but from the adverse effects profiles, you wouldn't expect that to be the case. I'm quite a big fan of asking patients at the end whether they know which group they were allocated to, but we didn't do that in the phase 2 study.

DR. MCMAHON: The second question is a bit more scientific. And that's, do you know whether the compound is CNS penetrant, may have other actions that you haven't explored?

DR. RICE: In rat, anyway, it doesn't penetrate into the CNS. It's peripherally restricted.

DR. CLARK: I noticed one of the outcomes that you looked at was spontaneous pain in the clinical study and I think the result was positive.

DR. RICE: Yes.

DR. CLARK: In the pre-clinical models, did you happen to look at spontaneous pain, and do you think it would correlate?

DR. RICE: Yes. Whether we can measure

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spontaneous pain at all in animal models is another issue. And Frank has already stood up. You want to talk?

DR. PORRECA: No, not for that. I had a different question.

DR. RICE: So my take on that is we look for changes in ethologically relevant behaviors. And we looked in a paradigm that is thigmotaxis in the open field. It's essentially predator avoidance behavior, and the hypothesis, animals that are in pain, would engage in increased predator avoidance behavior.

We did that in two of the pre-clinical studies and the signals were quite interesting. The first one, we did see a signal at the intermediate doses, but at the higher doses, we didn't, and I'll explain that.

With EMA401, we didn't see a signal in that regard. And we think the reason is that we made observations with both drugs that the injections were highly -- we used intraperitoneal dosing, and the injections themselves were aversive. We think

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it was a pH effect from the solution. And I don't want to use the word anxiety, but the anxiety related to that injection immediately prior to that may have confounded it, so it's picking up a signal in other regards. But in the EMA300 studies, we did see something of a signal in that regard. But they're not conclusive. That's why I didn't put them up.

So we did look for what might be a surrogate measure of spontaneous pain in animal models, but as we all know, we've got a long way to go in that regard.

DR. PORRECA: That was a very, very interesting story and very exciting. I am not really sure I understand the mechanism, I have to say, so that's one thing that I think seems mysterious. You were going kind of fast with he slides, so I didn't see the selectivity of the molecules for 82 versus 81. I know it was probably up there, but maybe I didn't catch it.

Then the other question related to this is, what is the interaction of these molecules with

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other targets? So ultimately, I guess what I'm asking is how do you know that the effect that you're producing, that you're seeing, is due to this 82 mechanism? How can that be? Is that established or what?

DR. RICE: Here is the selectivity data. But we hadn't put it in for other targets. As far as we know, it isn't interacting with other targets, but I don't think we can be fully confident in that.

DR. PORRECA: Yes. So how can you demonstrate that it is this mechanism, I guess?

DR. RICE: Yes. So there was the 82 knock-out.

DR. PORRECA: But that was affecting the actions of angiotensin, right?

DR. RICE: No, no. The effect of the drug is abolished in 82 knock-out animals in CCI. So they put that one up. But in terms of the screening we've done so far, we don't think it's active in other sites, but it's not really my area of expertise.

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DR. KATZ: Andrew, nice presentation. I'm curious whether you guys made any effort to subtype the patients with PHN, because if you believe in typology of PHN, you could imagine that a peripherally restricted compound might not work on the deafferentation subtype, which is present supposedly in a minority of patients.

DR. RICE: We didn't and we discussed it extensively before initiating that trial. Remember, it's a phase 2 study, so it's a small trial. And at that time, we were engaged with the German Neuropathic Pain Network, QST profiling, which we do in our group. It takes probably one and a half hours per patient. It would be very costly to supply all the centers with the equipment, and to train them, and ensure data validity.

That was before Roy has published his experiences with the Pfizer, sort of, if you like, bedside QST profiling. And at the moment, we are currently discussing whether we should include that in the next study. My personal view is that we

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should.

There is also a paper that has come out of the IMI Euro Pain Network, that I think is going to be a landmark paper. It comes from Charles Jenson and Nana Finrap. It's still under review, so I don't want to say too much about it. But they have fairly conclusively shown that, in a mixed group of patients with peripheral nerve injury, PHN, and diabetic neuropathy, using the full German QST profiling, patients who you would allocate to sensory gain phenomena respond to oxcarbazepine, and those that don't have a sensory loss profile do not. To me, that is a very important paper. It's a paper we've been asking for in our field.

So the short answer is, we didn't include it for this study. We did think about it. But the barriers to getting robust sentry phenotyping data were not conducive to a phase 2 study. We're actively talking about it for the next study. It's one of the things we're thinking about.

DR. KATZ: Thanks.

DR. BASBAUM: Last question. I am trying to

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get a sense of the patients. Some of them were still on meds. Some weren't. Were they not on because they didn't work? And in the results, if you look at the results, rather than an average effect, those that were still on gabapentin, for example, did they have a better effect?

What I'm really getting at in a more general question is, does a patient that responds to gabapentin also respond to duloxetine, also respond to this antagonist, or are they completely unpredictable?

DR. RICE: We saw no such effect, whether they were on treatment or off treatment. But this trial wasn't designed to look at that. It's probably too small to look at that. And the numbers of patients on any one drug or any one class of drug wouldn't really be enough to conclude much from that.

DR. BASBAUM: But many of the patients, the great majority, were on nothing --

DR. RICE: Yes.

DR. BASBAUM: -- which seems surprising.

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DR. RICE: Not when you consider that they were recruited in eastern Europe. Many of the drugs that are currently available are not available there.

DR. BASBAUM: Good point.

DR. RICE: Even for South Africa, a lot of the drugs are not available.

DR. TURK: Roy, you want to get the last word in this session? There will be an opportunity for a panel, so don't feel, anybody who wants to come up, that we didn't let you. Roy?

DR. FREEMAN: So maybe three quick questions, 1A, B, and C. So 1A, Allan asked me a question yesterday about criteria for moving from pre-clinical to clinical. And here, you are presenting, I guess -- and I've put Tom in this position once before -- the poster child of the move from bench to bedside. What clues do we get from the decisions that were made at the pre-clinical stage as to when to move to the bedside, 1A?

1B, and I'll just ask two. There is a, I

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don't know, growing conventional wisdom that neuropathic pain trials should be done in western Europe or the U.S.A. This really confronts that conventional wisdom square in the face. And it confronts it in a way that is somewhat surprising. It actually shows that the placebo response was attenuate.

Your effect size -- I think those of us who look at these trials pretty frequently -- was driven by the attenuated placebo response that occurred in the second half of the trial.

So comment on those two questions.

DR. RICE: So before giving this presentation, I was talking with Tom and various other people about what differentiates this pre-clinical program from many others. And actually, there are some bells and whistles that prevented the human DRG studies, for example, that gave us confidence.

We replicated some of the pre-clinical model findings in independent labs, but that's actually not fundamentally different from many other drug

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development programs that have not had success in phase 2. And the other thing to emphasize is, we're still only in phase 2. We've got phase 3 to go.

So I think the problem with pre-clinical development programs is they are great at identifying the drugs that do work, but they're not good at identifying the drugs that don't work. So to a degree, I don't want to use the word "luck," but we're using a drug that now is shown to have efficacy in phase 2. We didn't know that when we were doing the pre-clinical studies. But there's nothing that really differentiates the pre-clinical studies, that I can see, that is fundamentally different from any other drug development strategies.

Eastern Europe. I don't share your conventional wisdom, actually. I think one of the problems with doing studies in the U.S. and Europe is you tend to recruit from secondary care or even professional patients and professional research centers. And you often then attract the

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treatment-resistant patients who haven't responded to anybody else. And that's why in the U.K., we are piloting -- recruiting patients direct from primary care using the electronic healthcare records that, for example, cover the whole of London or Scotland.

So one of the reasons we went to eastern Europe is, of course, cost, but also we are more likely to recruit a population that might, in a way reflect a truer PHN population, if you like, than those that have been through a lot of treatments that are available in the U.K. and Europe that are probably treatment resistant. So that was one of the rationales.

I think that would be an interesting point for the discussion about the geographical location of studies. I think Bob has talked a couple of times about some of the problems related to becoming professional patients in trials.

Can I just make one other point? It's actually not in this study, but it is something I would like to do in another trial. We've got a

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paper in press on the Pain website, which is a negative trial of pregabalin in HIV neuropathy.

An important thing in that study, although it didn't make any difference to the negative outcome, was that we randomly sampled the patients because it's the culture in HIV for drug sharing, for plasma levels of pregabalin. And we found that an appreciable number of patients allocated to pregabalin, despite all the normal controls for adherence, had no detectible pregabalin in their blood. Con-meds were found more often than we expected, and some patients allocated to placebo had detectible pregabalin in their blood.

I think that's something that isn't reported in clinical trials more often and probably something we should be taking more notice of.

(Applause.)

DR. TURK: Thank you for that great presentation. And again, to stimulate us to be thinking of the trials we're doing, and advancing trials, and moving things forward, the next presentation is going to be along the same vein,

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although we're talking about something that's a little bit different, of course, with a different molecule.

We'll be talking about anti-NGF antibodies, and Nat Katz is going to be presenting to us. Many of you know Nat. He's been involved with analgesia solutions for a long time. He's also affiliated with Tufts University and has been for many years. And I'll just thank him for all the assistance he's given us over the years to ACTTION IMPACT. So thank you very much, Nat.

Nat is going to start giving you another story about how a molecule moved forward, with the idea being, again, we're trying to be on this, where are we seeing some positive findings to maybe balance some of the concerns or some of the negative that we've been hearing or have been hearing for a number of years.

So Nat, they're all yours.

Presentation – Nathaniel Katz

DR. KATZ: Thanks very much, Dennis, and thanks to the conference organizers. It turns out

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that my talk follows up perfectly from Andrew's and, more particularly, perfectly from Roy's question 1A to Andrew. And I think, if I had more time, I would have retitled my talk, "An Answer to Roy's Question 1A."

(Laughter.)

DR. KATZ: The only reason I agreed to give this presentation is because I know that probably half the people in the room have been involved in the anti-NGF story much more intimately than I have and probably know it better.

But I thought it would be a good opportunity for me to go back and look at what I think is probably the most definitive success story that we have in translation of efficacy. With the anti-NGF antibodies, we now have more than 30 trials, more than 10,000 patients that have been studied.

So the clinical efficacy signal is incontrovertible. There's really nothing to talk about there, except if you want to talk about how massive it is. And of course, there is a strong pre-clinical signal as well.

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So the reason I agreed to give this talk is to try to figure out, at least in my own mind, what might be an answer to Roy's question 1A. What does differentiate a true success story like this, from an efficacy translation perspective, from all of the other compounds that I and others have been giving to people for decades, that seem, at least on the surface, to look similar from a pre-clinical perspective, but of course have not worked out yet in patients.

In terms of disclosures, I have consulted extensively for, first, Rinat, and then Pfizer, and also J&J on their anti-NGF programs. I also worked for virtually every other company in the analgesic space at one time or another. However, I do not have any long-term stake in these compounds or any other compounds, actually, in terms of patents, royalties, equity, et cetera.

So it's been stated many times that rational analgesic drug development, in terms of trying to find new compounds for new targets, has not worked. And the anti-NGF antibodies is clearly the most

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definitive exception with due deference to the story that Andrew told a minute ago and the one that we'll hear momentarily.

So again, my purpose is to try to figure out if there really is anything different about the pre-clinical efficacy profile that was seen at the time that the decisions were made with this class to carry them forward into clinical trials that gave extra confidence.

Now, I will focus just on one of this class, which is tanezumab, which used to be called RN624, purely for my own personal convenience. I didn't have the time to look into the origins and data of all the products in this class. And for the purpose of today's presentation, I'm just going to make the assumption that this product represents the class. If that turns out to be wrong, then I'll be wrong, and I'll have to give a revised version of this presentation sometime in the future.

Anything that I have to say that is intelligent, you can attribute to this guy over

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here. You can see him walking around the room. His name is Dave Shelton. He's sitting in the back. And Dave has been intimately involved in this whole story, and has witnessed, and has continued to witness, the living history of the development of this class of compounds from the very beginning.

So he has been very generous in spending a lot of time with me, helping me get these stories straight myself in making sure I understand the concepts, especially on the pre-clinical side, which I never present pre-clinical data because it's not my thing. So only because of Dave's collaboration do I have the confidence to present you a little bit.

Another person who has very generously spent some time allowing me to interview her is Pat Walicke. Pat Walicke gave me a call in 2004 and said, "We have this interesting class of compounds. We think it might work for pain," the same question I get asked every day, "What kind of clinical trial do you think we should do?"

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So Pat ran the program, the early clinical program on tanezumab and spent some time with me over the last few days telling me what was going through her mind as she was making decisions for this program.

Finally, Leslie Tive, who many of you probably know, who is currently at Pfizer and currently has a key position in this program, also provided me a lot of insight and information.

So I'm going to tell this story in seven chapters. First chapter, at least the way that I've chosen to divide it up, is that nerve growth factor itself was very well known. There was a very well-established science going back to the 1950s as the first in the neurotrophin family. And its main role had been perceived as being growth and survival of neurons, neural structures in the context of development.

Then data emerged in the 1980s that not only did NGF have a role during development, but it also appeared to have a role in inflammation, where it was dramatically upregulated. That paper came out

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in 1987 and that observation actually just sat there, that NGF seems to have a role in inflammation as well.

This is actually the paper that I'm referring to, Weskamp and Otten, 1987. And as you can see, it was interesting to me -- I've not really been intimately involved in this field at all -- that looking back at this paper as being one of the progenitors of the whole notion that NGF might have some involvement in pain, you can't even find that in the title of the paper.

ELISA for nerve growth factor, they were basically developing a tool for studying it. But if you look at the paper itself, you can see data showing that NGF is dramatically upregulated. This is a blister-based preparation here. This is intraplural injection of carrageenan here, where there was this very major finding of regulation of NGF and inflammation.

So that observation was sitting there. And then, at the same time -- I will call this chapter 2, and I've titled this chapter, "NGF

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Hurts." And so in the early 1990s, two observations happened at more or less the same time. The first is that NGF was being developed by Genentech at this time. And I know there's some folks from Genentech in the room, and maybe they can pipe in after, too, and fill in anything that I leave out.

NGF itself, because of its role in promoting and supporting neural growth, was being developed as a therapeutic treatment for diabetic neuropathy. There were some laboratory findings that suggested that it could be beneficial, and then there were first some phase 1 and then some phase 2 studies. And the observation was that, when you injected NGF into people, it hurt. And I'll tell you more about that in a minute.

The second set of observations occurring at around the same time is that the same thing was happening in animals. If you injected NGF into a rat, it hurt. And I know that there are people in the room that were involved in that, and I'll look forward to any further insights you have about that

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as it evolved.

This is just a quote from one of the papers of one of the two phase 1 studies that was done on NGF itself. And I think it's worth listening to this because it gives you a flavor from a clinical perspective how unusual and how intense and provocative this observation was.

"Subjects who received recombinant human NGF developed diffuse myalgias beginning about 60 to 90 minutes after administration, worsening over the next 4 to 6 hours, and then slowly resolving over 2 to 8 days.

"The subjects reported mild to moderate pain with swallowing, pain in the masseter muscles, increased by chewing, sore throat, and pain with eye movements. You never see anything like this in a clinical trial.

"Sometimes, abdominal and limb muscles were involved. These myalgias were described as though one had run a marathon without preconditioning, or as though one had done a thousand sit-ups or push-ups."

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That was a quote from one of the patients. I've got more quotes, too, but I cut them out for the interest of time. This is a very stunning observation, I would say, from someone who does clinical trials.

So that's just a piece to give you a flavor of what was going on in the clinical side. The same observations were made in the other phase 1, the phase 2, and then finally the phase 3 study of NGF before, finally, that program was stopped because of failure of the phase 3 study.

Then on the pre-clinical side, you've got another paper showing that the same thing applies to the rat. And here is his paper from Lewin and Mendell in 1993. And this is just one of the many tables and figures in that paper that gives you a sense for, if you give NGF to rats, you get this major mechanical hyperalgesia, also a thermal hyperalgesia compared to the control rats.

There are many other similar convergent findings, and the interpretation of the authors was that administration of NGF can lead to a profound

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behavioral hyperalgesia, similar to what we were seeing in the patients.

The next chapter in this story is that, well, if NGF provokes pain, why don't we give some kind of anti-NGF and see if that blocks pain? That would be the next logical step in the story. So there were three main research groups that were working in this area, and there are folks in the room who were involved in those studies. And essentially, the findings were that, if you do a model of inflammation in rats and you give anti-NGF either before, or even during, or after the induction of the inflammation, you prevent the hyperalgesia.

However, interestingly, you don't modify the science of inflammation. And this was one of the interesting findings early on, that although this is being given in the context of inflammatory model, you're modifying the pain behavior without modifying the manifestations of inflammation.

I think it's also worth noting that this finding was convergent across three different labs,

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different continents, different investigators, different countries, different approaches to neutralizing NGF; and I would imagine, if we went into it in more detail, different strains, different feed, different bedding, different this, different that.

So part of what we'll get to later when we talk about what seemed to be the hallmarks of robust clinical data -- and this follows directly on Andrew's comment -- this sort of convergence of findings that are robust across multiple conditions may be one of those clues.

This just happens to be the paper that Clifford and his group published of the three. I just pulled it out because Clifford wasn't here to disagree with how I would present it. And here is just the sensitivity score from one of these models comparing the CFA injection to the CFA plus the NGF, showing a substantial reduction in the sensitivity.

So scientific research doesn't happen in a vacuum. It always happens in a social context, and

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in a cultural context, and in a funding context. And so I think it's interesting to talk about what was going on that facilitated what seemed to be very rapid progress for across a few chapters of this story in a relatively short amount of time.

Again, a lot of this thinking comes from Dave Shelton. First, this wasn't brand new, although actually, interestingly, Andrew's science is kind of brand new. But one of the things that gave comfort at this time is that there was widespread experience with a concept of what NGF did.

For that reason, a lot of labs had materials lying around that they had relatively ready access to relatively rapidly initiate different kinds of experiments. Because of that, there was also dispersion of skills and materials in multiple different labs. And from what Dave indicated to me at the time in Genentech, there was very little experience in Genentech. There was no experience, actually, in developing an analgesic.

So the folks at Genentech saw value in

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collaboration with external labs, some of whom are in this room. And what facilitated those collaborations at the labs is that those labs were there with knowledge, experience, and materials ready to go.

Then finally, I think that it seems that the convergence between what was being seen pre-clinically and the pain provocation that was being seen in the human model clinically, that synergism of findings between the rat and the human also gave people more confidence to proceed.

Chapter 4, I called "Back in the Real World." And again, things happen in the context of funding. So what was going on in the real world while all of this elegant science was progressing? So Genentech was transitioning its focus to oncology and not pain, although rumor has it that there is a renewed focus on pain at Genentech, and that would be nice to hear more about now.

But back then, pain was being transitioned out until, finally, the entire Genentech neuroscience group was shut down, and there were

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folks that believed in the program, but the company had decided not to pursue it.

So Rinat Neuroscience was spun out in 2001 based on VC funding, and the main drivers of this new entity were the folks that you see here, Arnon Rosenthal, Patrick Lynn, and Alun Davies, although of course there are multiple others involved as well.

As Dave indicated to me, they looked at their new building just down the road from Genentech on September 11th, 2001, so a memorable day for a variety of different reasons, announced their funding two months later, and then they were in business, trying to figure out, "Now, what should we do with this? What should we spend our investors' money on?"

This is what determines what progresses. And so there were a variety of different factors that facilitated the decision to accelerate into a pain program, but there were a few obstacles as well. And I think it's interesting to think about these practicalities.

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They were a new VC-backed company. They wanted a clinical-stage molecule. That's what the investors wanted. They were very comfortable with antibodies. That was their sweet spot. The pre-clinical efficacy data was deemed at the time to be robust. And so we've just had a discussion of, "What does that mean? Is one pre-clinical package any more robust than another?"

On the next slide that I'll show you in a minute, I have done my best to define what robustness is in terms of what the perspective was at that time about the data. And I think that's one of my take-home messages from this presentation, is what does robustness mean? So you'll see that in a second.

There was a recognize at Rinat at the time that we can fool around all we want with pre-clinical models, but the only way to actually characterize the efficacy and safety of the compound is in the clinic. And finally, there was directionality for the pre-clinical program. There was somewhat better efficacy in one type of pain

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than other types of pain, and I'll tell you more about that later. But the conclusion of the group was that what they called deep somatic pain, arthritis models, that seemed to be where the strength in the pre-clinical program was.

It was very interesting to me to hear what the obstacles were, and this may astonish some of you as much as it astonished me. First of all, apparently, there was a lot of debate at the company at the time about whether -- the quote was, "How can we compete with aspirin?" was the quote. "Do we really need a new pain therapeutic?"

What I heard in my mind, what I said to myself in my mind, when I heard that was actually a question is, "Wow. How disconnected these companies are from the real world." How can you ask a question like that? You just have to walk into a pain clinic and spend 15 minutes there, and you know that aspirin is really not doing the job out there in the real world. Forget about the GI bleeds.

But that was actually a topic of discussion.

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And boy, if this is actually still a question in boardrooms of investors, then we really have a communication problem on our hands that we need to fix. I don't know if it is or it isn't, but if it is, we need to work on that.

Another interesting dimension of this decision-making process was a concern that, well, pain is a primary care problem. Everybody's got it. Is a biologic ever going to be a primary care drug? With the implication being that, "Unless we can really get it out there in primary care, where pain lives, it's not going to be successful in the marketplace."

On the other hand, there was exactly the opposite argument, which is that, well, what if we get some weird safety issue? Aren't we going to be glad if we start studying cancer pain or some more niche part of the population, where we can make a more compelling risk/benefit argument if there does happen to be some weird risk that we run across?

I think I'll leave that in your mind for later because it remains an interesting issue,

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given some of the safety questions that have been raised around this class of a product. And I will say a word about that later, although my focus will not be on safety during today's presentation.

So having said all that, the facilitators outweigh the obstacles. They decide that there was room for a new pain therapy, and the program proceeded.

Now, here is what I mean by robustness. So this is sort of a summary of the large amount of pre-clinical data that was available at that time. You saw efficacy. Regardless -- despite the fact that it was studied in different pain models, different endpoints within those models were all positive. It wasn't just the von Frey or just the radiant heat.

Virtually, whatever endpoint you looked at in those models was positive, different time points within, though. It wasn't just positive at hour 4, but it was positive across a spectrum of time points. There were different types of rats used in the different experiments. There were different

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types of experimental conditions that were used, different feed, different bedding, different researchers, et cetera.

Even the antibodies that were used in the different studies were different, providing again convergent validation of the mechanism, different molecules that are not anti-NGF antibodies. For example, molecules that blocked binding to track A also produced similar findings, providing more convergence, and again, labs, investigators, continence, and finally and importantly, although I put it at the end, is that there was consistent superiority against active comparators.

That was important to the team for, really, two different reasons that may sound the same, but they are different. One reason is that, if something really is better than morphine, gabapentin, et cetera, maybe you will be more likely to see technical success in your clinical trials if the drug's that good.

Then secondly, there's the commercial flip side of that coin, which is that not only might we

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eventually see p less than .05, but maybe out in the marketplace, we'll see the commercial differentiation that really is the requirement these days for successful commercialization, especially of expensive drugs.

So this, to me, is actually a conclusion slide, although it's buried in the middle of my presentation.

So what did the team do next? This is chapter 5. It seemed like there was a lot of data, although most of it was out there in the literature, rather than having being done at Genentech or by some very closely held collaborator. And a lot of the models that were done, one can ask what the relevance is to human clinical pain.

So the next step that was taken was to confirm the pre-clinical efficacy profile with studies that were sparked by Genentech, but then conducted with the insight, and knowledge, and experience, and laboratory expertise of a number of different collaborators.

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So the key papers are the one from Tim Brennan's group of the hind paw incision model; a paper authored by Dave Shelton and collaborators with a more aggressive model of CFA, where it's injected into the tail base and produces a profound destruction of the joint; and then also a cancer bone pain model from Pat Mantyh's group that we've heard mentioned previously.

This is a picture of a cute little rat foot with an incision from the hind paw incision model and just a graph from the paper from Tim Brennan's group showing that the anti-NGF antibody brought your pain sensitivity just about to what it was after the sham procedure.

This is a picture from the bone pain study from Pat Mantyh and his group, and shows -- actually, there is a lot on this slide, and in the interest of time, I'm going to gloss over it.

First of all, when you put sarcoma cells in bone, in this model, you get this perfusion of an aroma in neurite formation. And that, as well as

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the pain associated with it, are blocked by the administration of NGF.

So that gave a very powerful convergence with the other pre-clinical findings. And all those three studies that I mentioned were positive, so done under much more careful conditions, and with more visibility to the sponsor at Genentech, and all convergently positive.

Chapter 6, in the meantime while all this is going on, nothing that I've showed you today -- I've not mentioned the word tanezumab yet because nothing that I've showed you has anything to do with tanezumab. It was not used in any of those studies. And so meanwhile, Genentech is trying to figure out what antibodies should we actually bring forward into the clinic.

I'm not going to go through this in any detail. Suffice it to say that there were a number of criteria that the company had for what would be an appropriate antibody among the many options that they had. And they finally ended up in April 2002 with the product that they had ultimately decided

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to bring forward. And if you have any questions about that, then we can ask Dave at the break.

The next chapter and the last chapter is where I personally got involved in this program, which is now, what clinical syndrome -- given this pre-clinical data, what do we do clinically. And there were a number of drivers of that decision. And, again, that program is led by Pat Walicke.

First is that I'm not actually showing you any details of the breadth of pre-clinical data, but it was studied in both somatic, and neuropathic, and visceral pain models. And while there were positive signals of efficacy across all three types of pain, the feeling the team was that the efficacy was most profound in the bone pain model.

So the pre-clinical was driving them towards bone pain. Because of funding, and the pressures, and the need for success, there was a drive to try to get early signals of efficacy from the phase 1 studies. And this is a question that I get asked all the time. We need to do phase 1. Is there any

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way we can get early signals of efficacy, of human efficacy, out of the phase 1 study, and what are the options?

The options are studying patients in phase 1 using human experimental pain models in your healthy volunteers, which, as much as they might show you interesting data, they raise all sorts of other questions about whether that itself is going to translate into patients when you get there. And that's pretty much it.

So the drive was to try to do phase 1 in patients. And that was not really an issue at a biologics company because, given all their experience in oncology, they were used to doing their phase 1 studies in patients anyway, so that felt very natural to them, although it might feel awkward in a small molecule environment.

So if we're going to study patients, what kind of patients? If we're interested in bone pain, then the feeling was that osteoarthritis is probably the most practical, and convenient, and representative model of bone pain. You can find

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the patients. There's lots of them out there. Their pain syndromes tend to be relatively stable. It's not like back pain, which is complicated for reasons that were discussed in detail yesterday during another talk.

So the decision was made to -- there had been lots of positive studies in various kinds of agents in osteoarthritis, relatively well-established how to do those studies, validated endpoints, et cetera, et cetera.

So the other interesting facet of their phase 1 studies, and what gave them an opportunity to capture early signals of efficacy, is that because of the prolonged half-life of these antibodies, you need weeks between dose escalation cohorts. And observing patients for weeks gives you more opportunity to capture efficacy data than if you're just observing a patient for a few hours with a chronic pain syndrome; although that can be done, too, which is another story. But at that time, that was viewed as a positive reason to do phase 1 in OA and try to get early signals of

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efficacy.

Now, to Pat Walicke's credit, she's a very smart woman and has a lot of experience in clinical research. She read a lot, and she caught on to this whole assay sensitivity concept. And back in 2004, it was still early days. Our first impact meeting was, what, 2002 or something like that? And so I was very gratified to hear from her on the phone the other day when I spoke to her, that she read all the IMPACT papers at that time, was very familiar with all the contributors. And it made me feel good that we had accomplished something without necessarily knowing it at that time.

So the phase 1 studies are not published, so I don't have that data to show you, but suffice it to say they were positive, as you probably could guess. And that led to proof of concept, a rather large landmark proof-of-concept study of tanezumab in patients with osteoarthritis of the knee. This was published in the New England Journal of Medicine in 2010 and showed essentially a degree of efficacy that had, to my mind, never been seen

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before in a clinical trial of an analgesic for any kind of chronic pain indication.

Just to give you a flavor, this is the placebo in this grayish color. And they had a 15-ish-millimeter reduction from baseline in their average pain intensity. And here, in the highest dose of the tanezumab group, you've got a 45 millimeter reduction in pain intensity, a 30 millimeter difference between active and placebo. And I can tell you, from the world of clinical trials, you just don't see that. You just don't see that.

So the rest kind of is a history. I'm not going to dwell in detail on what's happened since then, since this is a meeting about translation. And I think the focus is about, well, what was it about the pre-clinical data that might have been different?

I will give you a quick overview of where we are now with this particular compound. This is an overview of all the randomized control trials that have been done up until the point where this

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product was put on clinical hold by the FDA in 2012 because of a joint safety issue. So there has been very little done since 2012.

In fact, I think the only other studies that I've read out since then is the cancer study because that's the only study that was allowed to proceed at that time.

So here is what tanezumab does. So first of all, just in terms of the breadth of the program, you've got 30 clinical trials that have been completed, 28 plus 2 phase 1 studies that are not on this slide.

You've got 17 studies in osteoarthritis, 2 studies in chronic low back pain, the cancer study that I mentioned, a bunionectomy study, which was negative. And I've organized these studies by pain category. So the bone pain or the somatic pain are up here. There have been two small neuropathic pain studies and four small visceral pain studies.

The results, what these pluses and minuses mean are the following. Two pluses means that the

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p value of the study was less than .05. One plus means that the p value of the study was not less than .05, but there was a numerical trend favoring the active group. And the minus sign means that the study was flat. There was no sign of efficacy whatsoever.

So just to give you the quick overview, there's strong evidence of efficacy and osteoarthritis, 2 positive studies in chronic low back pain with 1500 patients in them. The bone cancer study was not statistically significant, but there was a trend in favor of the active group that manuscript is soon to be submitted into the literature.

The bunionectomy study, which is an acute pain study, that failed totally. There was no sign of efficacy of tanezumab. But for reasons that I don't understand, they had no positive control in that study, which is a mistake. You'd never do a bunionectomy study without a positive control. I'm not sure why. There must have been a reason, but I don't know what it is; and now, more than a decade

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or so later. But so far as we know, this drug was not effective in this acute pain model.

Interestingly, you've got statistically significant results in painful diabetic neuropathy and a very small 30-ish per-arm clinical trial. In another 30-ish per-arm post-herpetic neuralgia study, the p value was -- I forget -- .09 or something like that, with about a .6 or .7 difference. So I think, actually, this medication probably works in neuropathic pain as well.

Visceral pain is kind of an interesting story. So there was a very small interstitial cystitis study. And a lot of people view interstitial cystitis as a graveyard. Good luck getting a positive study of anything in that syndrome, but in fact, the study was positive, prostatitis, a trend, but negative p value.

They started a study in pancreatitis, but stopped it because of the clinical hold after two patients, and there was a negative endometriosis study. So I think, again, an encouraging signal in visceral pain as well.

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So that's where we are right now with tanezumab. In the interests of time, the only point that I'll make with this slide, which is a pooled analysis of all the OA studies, is that there is superiority over an active comparator, which in this case was naproxen. And in the world of pain research, we don't see superiority over active comparators.

Also, superiority of oxycodone in a clinical trial, another osteoarthritis clinical trial; again, that's good, although I do feel obligated to point out that oxycodone was not superior to placebo in this study. So there may have been technical issues with how it was dosed, but still, I sense that it's superior to an opioid comparator as well.

Again, my talk is not on safety, but I feel compelled to mention that these programs were put on clinical hold since 2012. And the two main concerns have been joint safety. That was how it all started, and also sympathetic nervous system safety has been another concern.

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So I have two conclusion slides. And these are the lessons that I think I've learned as a non-scientist, as a clinician and a clinical trialist, from looking back at this particular program and trying to ask myself the question, "What was different here, if anything?"

These are my thoughts about it, and, again, there are people out here that know much more about this program than I do, and I'll be curious to how people react to this.

First is, I can envision in my own mind a robustness checklist. And what I mean by robustness checklist is that slide that I showed before. When your efficacy is robust across all those different sorts of conditions, it's more persuasive than if it's not robust across those conditions. That's the first lesson that I have learned.

I have looked at a lot of pre-clinical data from the perspective of the clinic over the last 25 years of giving drugs to patients in clinical trials, and I don't think I've seen a package with

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this degree of robustness. I haven't looked back in the bowels of my computer to confirm that that's really true. I'll be curious what other people think. But this feels very different to me.

The second checklist that I could imagine using is what I would call a methodology checklist. And what I'm thinking there is the checklist that Andrew Rice and colleagues have contributed to the literature, where if studies are not done and with all the features that Andrew has shown on his slide -- randomization, blinding, et cetera, et cetera, et cetera -- they are hypothesis generating only.

Thirdly, benefit over standards, that's something worth looking out for in the pre-clinical data set. And of course, I have seen a lot of data that shows supposedly superiority over standards, but I would bet money that, looking back at all the data that I've seen, you would not see that superiority over standards replicated across that robustness checklist that I gave you.

Next, just a word of caution -- and this is

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really more Dave's thought -- that beware of interpreting results about superiority when you're studying a drug that was -- when you're using a model that was developed for another drug. So for example, if you were testing ibuprofen versus morphine in a CFA model, you might be tempted to say, "Well, look at this great new drug, ibuprofen. It's better than morphine." But, of course, that model preferentially shows the efficacy of NSAIDs over opioids.

So be careful about drawing superiority conclusions with certain models since the models themselves may bias you in favor of one class over another.

This is a point that I got from Steve McMahon -- I'll give you the American pronunciation of his last name for the purposes of today -- which maybe he'll want to discuss in more detail during the break, which is that -- and I didn't really appreciate this -- a number of our efficacy models; if the drug that we're testing produces sedation, or dizziness, or nausea, or Lord knows what, the

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readout might be an analgesic readout. You might interpret that sedation as analgesia.

I actually hadn't ever thought about that before, but that makes me very skeptical now about models where a side effect might actually confound the primary efficacy endpoint. And I'm going to have to think a little bit more about which models ought to be viewed more skeptically because of that problem or, alternatively, what models should be viewed favorably because the side effects do not confound the readout of the primary efficacy endpoint.

So this is what I think I'm going to be looking for in the future when someone presents me a pre-clinical data set and says, "Do you think we should bring this into patients?"

From a clinical perspective, I think there are also some interesting lessons to be derived from this story. But one lesson is that, now that we have methods for improved assay sensitivity, which we could present a long list of what those are, maybe we should be doing more phase 1 studies

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in patients and utilizing those methods to try to get more early signals of efficacy. And I see that happening in pain research from my vantage point.

I think that if the phase 1 studies had been done on volunteers and there was some readout on experimental pain, that would not have been persuasive as what they had, which was a readout in real patients. So I'm a fan of studying patients and getting clinical endpoints when you can as opposed to experimental endpoints, although hopefully we'll learn more about those over time.

The methods to augment assay sensitivity are really critical, especially in the early small studies. It seemed like, in this case, there was some pre-clinical suggestion about where the most robust findings were. And that does seem, at least from what we can tell right now, to map onto what we're seeing clinically.

Finally, you don't know what your clinical efficacy profile is until you study your drug in patients with different clinical syndromes. There's just no shortcut. And I think it's

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fabulous that Pfizer, J&J, and other people that are working on these compounds have the courage and vision to test diverse clinical syndromes, even where we actually don't know a lot about how to do clinical trials.

Who knows how to do a chronic prostatitis study? If you raise your hand, you're a liar. Nobody knows how to do those studies. And so I give the sponsor credit for at least beginning the process of exploring how to do studies in clinical syndrome, where there is a high unmet need, but not a lot of track record about how to do the studies.

That minor point there is that we and the clinical research methods development community need to develop better visceral pain models to foster research in that area. Thank you very much for your attention.

(Applause.)

DR. TURK: [Inaudible – off mic] -- stimulating to get us again thinking about, what are the things we want to be thinking about as different molecules potentially move forward. We

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are running a little behind schedule, so we'll just take a couple questions. Then we'll save other ones for the discussion.

Sharon?

DR. HERTZ: Sharon Hertz. So you presented a lot, and you really focused on the efficacy. But we can't escape thinking about the safety. And this is a really good example of situations that arise.

So two points that I'd like to make is there's been a lot of discussion about how dirty molecules work better, but they also hurt more. And we still have some pretty notable safety issues that need to be addressed with this. And what's interesting from a translation perspective is how the efficacy here seems to have been very predictive, with the safety not so much. And that's something that would be interesting to see if there's any way to think about that and anticipate it more in the future.

There was one other point I wanted to make, but I think I lost it already. But anyway, I don't

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know what else could have been done. We have looked back at the non-clinical work, trying to see if there's any way that we could have anticipated the arthropathy signal. I'm not sure that there's anything there that would have anticipated that.

But to the extent that we don't have them yet, I am still a fan of more specific targeting, even if it's not an expected target, in terms of reducing the amount of noise in the safety side of things.

DR. KATZ: What do you mean by more specific targeting, Sharon?

DR. HERTZ: So even though this is called nerve growth factor and we're targeting anybody who's against it, it's not specific for nerves or nerve growth in any way. And that's where we're starting to see the other -- some of this toxicity profile is nerve related, but it's also not nerve related. And apparently, if you start really exploring the targets or the areas where nerve growth factor are active, it's pretty diverse, which then gives you the opportunity for many

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potential safety signals, including ones that aren't anticipated.

DR. KATZ: It's a very interesting point, and I also took note of the fact that NGF inhibition produces a cascade of all sorts of different effects. And the question is, well, is that positive because it's necessary? Maybe that's a way to produce better efficacy from new compounds rather than narrow targeting. But maybe that creates safety risks.

Chas?

DR. BOUNTRA: Nat, I thought that was brilliant. Thank you. Just a couple of comments. It's interesting -- I mean, all the data that Steve and Clifford generated in the mid-90s. I mean, everybody was aware of that. But it was interesting that big PhRMA weren't leaders in this target. Secondly, both the two stories we've heard today, this afternoon, have been peripheral mechanisms.

Thirdly, you seem to be implying that, if you have a target or an asset that works in loads

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of animal models, treating pain almost as one big thing, it's going to work in all pains in the clinic. I'm not sure I agree with that. I also am not sure I agree that the data package for this is more robust than everything else we've taken into the clinic.

DR. KATZ: I think that's a question. It's a latter point that you made, Chas, is this pre-clinical efficacy profile really more robust in the ways that I suggested than other compounds that have been brought into the clinic and have been shown in clinical trials to fail?

I personally think that, if anything were to come out of this meeting, that would be something very important to look at, because if there is a way of predicting in advance what products are going to work in the clinic and what aren't, by looking at the pre-clinical efficacy profile, if my intuition is right, that's worth knowing. And if it's wrong, it's also worth knowing.

DR. TURK: We're going to have the last question this session from Allan.

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DR. BASBAUM: I'm just curious. I'm very familiar with the program. The discussion is, you seem to be convinced, in retrospect, it's correct, of course, that OA was a good indication to go after. And I don't know -- and Dave Shelton would know the answer to this. I don't know if it was ever tested in the MIA model, the monoiodoacetate model that's ostensibly an arthritis model.

Then, of course, the real issue is, is OA, even though it responds to an NSAID, an inflammatory condition. And one wonders whether that choice in retrospect was great, but really, if one had to sit around a table, would one say, "Given that pre-clinical package, let's go after OA."

Deep somatic pain in OA, I don't find those necessarily the same.

DR. KATZ: So obviously, I don't know the answer to your question, but I'll throw out an idea. I think, because none of these pre-clinical models are models of OA, the more convergence you have from different poorly representative models,

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the more robust the overall conclusion is that will ultimately work in some human.

DR. BASBAUM: What would you say about post-op?

DR. KATZ: What would I say about post-op in terms of what?

DR. BASBAUM: [Inaudible – off mic.]

DR. KATZ: Why didn't it work in the bunionectomy study, do you mean?

DR. BASBAUM: [Inaudible – off mic.]

DR. KATZ: I don't know. And Dave and I actually have spoke at length about that. And I don't know why. But I think -- Dave, maybe you remember. I think that the drug was dosed pretty close to the actual time of the surgery.

If there needs to be some time for these anti-NGF antibodies to do whatever they do, then perhaps dosing further in advance of surgery would be a strategy where it could have worked. Or it could be that the other idea that we discussed was that maybe whatever's going on early after a somatic insult of one kind of another is not

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dependent upon these NGF-related mechanisms, and it's only what's going on after some period of time, 12 hours, 24 hours, whatever, that becomes dependent upon NGF mechanisms.

So I don't know the answer, but there may be an answer.

DR. BASBAUM: That's a good question.

DR. SHELTON: Just to add one thing, that was probably more properly called a broken study. We went to sort of great pains to use one surgeon and be very careful that the surgeries were all very similar. And what we ended up with is patients with essentially pain scores of zero within 24 hours. So to be clear, we saw nothing that looked like efficacy, but we didn't have a lot to work with.

DR. TURK: Thank you, Nat. And there will be time again for more questions after, when we have the panel discussion. So if you've had questions for any of the speakers, we'll be bringing that back up. So we're continuing in the same pattern, which is to be looking at stories to

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tell us about how things have evolved. And, hopefully, at the end, you'll be able to start pulling some things together. And maybe you can give us some guidance and some advice on future molecules and future development of those.

Our next speaker is going to be Dr. Lars Edvinsson -- I'm probably saying your name wrong -- who is a professor and chairman of the department of medicine at Lund University in Lund, Sweden. It's my pleasure to have him come and join us. He's another one of these people who's traveled a great distance for coming here.

So now, we're going to be hearing about CGRP and blocking agents with potential for antimigraine therapy.

Presentation – Lars Edvinsson

DR. EDVINSSON: Thank you very much for the invitation to come here and speak to you. It's been a fabulous two days for me. A migraine is a very common disease. Perhaps up to 1 out of 6 people here in this room have migraine. And what does it characterize? Well, it's pain, moderate to

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severe pain, lasting between 4 and 72 hours, associated with a lot symptoms that you are familiar with.

The socioeconomic costs of migraine is astronomical. It's actually in the same level as a stroke. I didn't know that, so it's a very important issue to care for. I put on this girl here because we didn't qualify for the world championship, but in soccer for women, we are very good. And actually, she has a migraine.

There is a good match between blood flow in the brain and neurology symptoms. And as we can see here, this is blood flow in the brain. This patient has a visual aura. The blood flow in the visual cortex is low, coupling between the neuronal symptoms and flow of metabolism.

This patient has a visual aura that continues to be a sensory motor aura with the numbness and weakness of the arm. So it's a nice coupling. And another issue is just, you can say, some kind of a background because I would like to tell you that migraine is a disease of the central

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nervous system.

In a German study, they looked on patients with acute migraine attacks, and they had to come to the clinic in the middle of the night and they put on the PET camera.

What happened was that in these 9 patients, they had activation in the brain stem, in a region which is around, shall we say, the rough and the PAG. And also a very other notable finding, which has been not discussed so much, is this one. It's actually in the cerebellum.

Today's findings, we have seen that the purkinje cells of the cerebellum are heavy with CGRP, and the dendrites and axons are full of their CGRP receptors. So that's one issue we are looking into.

Another interesting aspect here is that, after treating these patients with sumatriptan, the pain disappeared, but these changes remained. So the attack was still there. And some of Peter Goadsby's work adds further to this that brain stem and other areas, like the hypothalamus, are

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involved in these attacks. So I'm just giving you this aspect. It's a CNS disorder.

So what am I doing here? Well, I am going to speak to you about my part of this kind of puzzle. And my original work was centered around understanding cerebral blood flow, its regulation in health and disease. And as we can see here, we have one brain vessel. We have the sympathetic nerves with, like, three signal substances. We have the parasympathetics with a lot of other signals like nitric oxide, acetylcholine, PACA, and VIP, and finally the trigeminal system, again, with many substances, including CGRP.

So why I tell you this is because we were doing all this kind of work as the first in the world, and we looked upon how these signals can regulate blood flow and their role. But the story about the CGRP came when the CALCA gene was mapped, the calcitonin gene, in 1983. Immediately when that was published, we made antibodies, set up Regimmune assays, and we had a whole lot of methods to examine this in the cerebral circulation.

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For example, like we have seen here with immunogold methods, antibodies towards, in this case, neuro peptide Y in sympathetics and here in the sensory, the large vesicles containing CGRP and the electron-lucent ones, presumably substance P at that time.

So that was a neat method. We also saw these kind of thin fibers in the blood vessels of the brain and also in other cranial vessels. We did tracing, anterograde and retrograde, and found that it comes from the trigeminal ganglion. And again, co-localization with substance P, which was the agent that was very hot for pain in those days.

The question we had at that time was to find out what are these sensory fibers doing for the cerebral circulation? At that time, we were not interested in migraine. We were just interested in how do these fibers regulate blood flow? And we did a lot of experiments, I can tell you, and different kinds.

This is, you can say, a cortical vision on top of a CAT, and you can measure the diameter of

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the PL vessels. We can apply a different kind of stimuli. And we tried a lot of vasodilators. We tried vasoconstrictors, only vasoconstrictors caused click contraction, like in this case, noradrenaline, and then returned very quickly to control. And that is to be expected, of course.

But in animals, where we had taken away the trigeminal nerve, innervated the sensory system. We got the same amount of constriction, but it took a long way to get back. So this was a trigeminal vascular pathway that we had found.

What is a bit funny for the migraine field was that people give vasodilators, and that causes a migraine or a headache-like symptom today. And I don't think that is activating this. What can activate it? We went actually into stroke models to look on that. And in patients with subarachnoid hemorrhage, this mechanism is activated to defend the brain towards vasoconstriction.

I also went to the pathology and pulled out the vessels from patients that have died from a subarachnoid hemorrhage, and it seemed that they

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were completely devoid of CGRP, but not of any other signal substance. So the theory worked.

So that's kind of where I think this mechanism comes into play, shall we say, in this defense role. It could, of course -- you talk about spreading depression in the migraine field. That is a kind of vasoconstrictor that you can elicit. And the aura is thought to be such a vasoconstrictor. And it has been shown that CGRP is part of this early hyperemic phase in spreading depression.

Now, if we now move into the CGRP receptor, this first part is something Frank asked me to do to give us a little bit of the history. Now, the more modern thing is that the CGRP receptor, as you see here, consists of this part, the 70 calcitonin receptor-like receptor, CLR, and ramp 1. When CGRP comes here, it docks here and this moves together, activating receptor, and recombinant protein, and then the G protein to produce its effect.

When we looked, where could the receptors be, because that's where the questions are today

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for the treatment of migraine -- at some time, it was a very large discussion. How about the endothelium? Is there any kind of CGRP receptors in the endothelium? And as you can see here, this is just a marker of the endothelium. And if you look on CLR or ramp, there is none.

So in other experiments, we showed it has nothing to do with the endothelium. The receptors are on the smooth muscle cells. And you can see, actually, they're all ramp 1, 2, and 3. But the experiments have shown that, if ramp is present, it predominates, so ramp 1 and CLR joins to form this receptor complex.

You have seen other kinds of antibodies. You can see, again, co-localization on the smooth muscle cells. They are recent, specific, ramp 1 and CLR antibodies.

If we compare human middle meningeal artery, that's a dura mater artery which lacks the blood-brain barrier and then middle cerebral artery, we can easily go down here and say that CGRP is 10 times more potent on the cerebral

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artery. The reason I think is due to number of CGRP receptors available in these vessels.

The dura mater has been used in a lot of migraine models as a surrogate. And since you are paying the researchers, you are well aware of C fibers, A delta, and the other kind of A fibers.

We find that in the dura, we have thin, unmyelinated C fibers and they contain CGRP. We have the A fibers, and they contain ramp 1 and CLR. So there's a clear separation between their localization.

Another issue is the mast cells, which have been discussed a lot. We can just move up to this one. There are lots of mast cells on the dura mater;' virtually none in the brain. And in rodents, they contain the CGRP receptor complex, CLR and ramp 1. You can get them stimulated, and they release histamine, which is one of the signals.

However, we have done it in humans as well. And then we don't find a receptor. And we have done experiments. We can't get CGRP to release

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histamine from the human muscles. So again, as people have been saying here, man is our key model. Strange, isn't it?

Here is a picture that Peter Goadsby gave me. So you can figure out that I did a lot of this kind of work since three decades. And at this meeting -- actually, it was '86. Peter came to Lund, to a meeting we had. And we met there for the first time. He was a young researcher working in Sydney. So we agreed upon collaboration and that's perhaps what started his career. I can always be nice to friends.

Very early, we did a kind of study where we stimulated a trigeminal ganglion in patients with trigeminal neuralgia. And what happened was that each patient secreted CGRP and substance P. That was to be expected. Both peptides are in the trigeminal ganglion, so no strange thing.

The key issue, what we did in all these experiments, was that we compared -- initially, if we take the samples from the external jugular vein or from the cubital fossa, it turned out very

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quickly that we never got any signal when we took the peptide measurements in the cubital fossa. And of course, it's because it's released from the hip.

In the migraine patients, it was also a very strange finding. CGRP was released in all the migraine patients, and it correlated with the pain of the patients. After triptan treatment, it normalized, and the pain disappeared.

At the same time, we did substance P and we'd never saw any substance P release. And actually, subsequently, I've taken samples and done studies of 10 more of the neural transmitters related to the cranial circulation, and it seems to be very unique that's it's CGRP that is released. For example, you can see again substance P.

VIP has a special story, and VIP is co-released in cluster headache, what is now cluster headache. Well, if you have a colleague that has migraine, and they have an attack, they have, of course, a severe headache, they are pale in the face bilaterally, so to say. A cluster headache, they have unilateral reddening. They

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have rhinorrhea, nasal congestion, other symptoms from the parasympathetics.

So we see this is from a migraine, but in those cluster patients, there is a co-release of VIP, as you can see, and also in another kind of chronic paroxysmal headache. The interesting issue with a cluster headache is that triptans work also on these. So there is a kind of brain stem interaction between the two systems.

Now, we move into something perhaps a little more interesting for you as general pain researchers. We have these small molecules. They are called gepants, and they have been tried in acute migraine trials. Patients having an acute migraine attack, they get a tablet, or sometimes injection with olcegepant, which was the first one. That caused the pain to be less, significantly less, so they work.

But that is not always the issue. If you like, you can just look at my review in the Lancet about all these results. But the burning issue is, where can they act? And of course, do they need

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the blood-brain barrier? And then we have the issues of antibodies and these Spiegelmers, which are another group of agents which can block CGRP.

I'll try to dissect the kind of parts that we need to discuss to understand where they could act because we know if they act. If we just take telcagepant, which is the one that is mostly studied, you can see that this is CGRP dilatation. And this is a human brain vessel gotten from neurosurgery. So that's your English part of the cortex when that is removed -- sorry.

Now, they have the tumor or an epileptic focus, which they are operated for. And sometimes, there is a small vessel which we can use. So we can see, here's a nice parallel shift with telcagepant, and here is a middle meningeal artery. Same thing. We can calculate to dissociation constant. And of course, it's the same receptor, so the PA2 values are the same.

What you can see also, if you are interested in pharmacology, is that the first part is a parallel shift without dropping Emax, but later on,

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that's dropping Emax. So there's competition and then some non-competition in the higher doses. It doesn't matter so much for the patient.

Are there other parts where these blockers connect? Where are the receptors? And this is not for you do any kind of major thing, but this is just a list to show inside the brain, that there are lots of different sites where we have CGRP receptors available, and some of them are listed here. Of course, in PAG, there are colliculus, for example, and some other regions here and also in the cerebellum; so not many binding sites that it could act there [indiscernible]. Do they pass the blood-brain barrier?

Then this has been discussed ever since the triptans were introduced into the market. People have said yes or no. They have been saying patients get stiff when they get their sumatriptan or some kind of neurasthenia.

Nork [ph] did this study. They gave a PET tracer, which was marked for CGRP receptors. And as you can see, perhaps not so good, but here is

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the brain, and some small radiance in the brain stem, but the cerebellum lights up like a beacon. Then they gave systemically tercagepant in a dose which is used in the clinic. Nothing happened. It's the same amount of PET tracing, PET signal. Only when they gave about 10 times higher dose of tercagepant did they, as I say, get the initial PET tracer to disappear from the binding sides.

So it suggests, if anything, that if there is a little tercagepant passing into the brain, it does not have much to do with the efficacy of the drug. So this is my current view, that even this small molecule doesn't pass in, shall we say, a dose that is producing the clinical efficacy.

What other issues do we have, and where else could the CGRP blockers and antibodies work? Could it be on the dural extracranial vessels or, which I would be advocating for today, the trigeminal ganglion.

For the gepants, we now have five of them. They have no effect on the general circulation when you give them. Why? Probably, CGRP doesn't have

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any tone effect on the circulation. So it's a good disease that nothing happens until you have the specific pathway activated. So that's when it can do something.

How about the antibodies? We have now three antibodies towards CGRP. We have one antibody towards the CGRP receptor available. Are they effective? Well, I think, a month or ago or so, it was presented data for two of the CGRP antibodies, that they were effective in chronic migraine.

How specific are they? Well, they are very specific for CGRP. And they don't cross a reactor towards other members of the same family, like amylin, calcitonin, or adrenomedullin. The antibodies are directed towards the C terminal. It's also interesting, just like tercagepant, they have the better focus and, here, better binding to primates than to rodents, and they are active in picomole concentrations. It's been studied in Cal Sure [ph], and of course, CGRP induces cyclic AMP generation, and this can be blocked.

There's also been a study looking on the

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skin vasodilatation and on an increase in middle meningeal lottery diameter. And it was compared with one of the gepants and virtually showed that the antibody that they studied was effective, but it was slow or in onset, and it lasted much longer.

We have another group of agents, which are called CGRP-binding Spiegelmers, oligonucleotides. And it's been difficult for me, really. I followed it a little to understand what they do, but it appears from people working there that you have this CGRP molecule in the circulation and these, should I say, oligonucleotides often are the size of 10 marrow or something like that. They can bind on to CGRP in making a complex, which is hindered or blocked from having an effect on the receptor sides.

The first one that I got my hands on was NOX/C89. And we had some effects, and I'll show you them. So there is also others coming out from the same company. They haven't tried them in migraines as I know yet, but speaking with the Kirk [ph], it's -- you can say -- it's like a

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chemical entity, almost like an antibody, but in a different way, but doing the same job.

Topical application of this NOX/C89 could inhibit CGRP-induced dilatation. And when you came to other methods, like electrical stimulation, it took some time in the method. And as I wrote here, "It was significant, not until there had been 7 or 9 stimulations." So it seems that it takes a long time for it to act in vivo, at least. And more recently, there is even more specific NOX molecule appearing.

So just having a look on the trigeminal ganglion, you can say we have small- to medium-size ganglion cells, and they contain CGRP. But the large ganglion cells, they contain the CGRP receptor. Can they communicate as I indicated? These red balls, I put out. I think so because we have, also, this -- it's actually nerve fibers that you can find within the ganglion.

Another very important aspect is these cells, the satellite glial cells. And they are like glia in other places. They've formed around

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the neurons, and they also contain the CGRP receptor elements.

Another aspect, which you probably are very familiar with is that if you give Evans blue, which is an old dye, the whole rat is blue, but the brain is -- of course, if you haven't manipulated it, it's pale. If you take out the pineal, it's bluish because it has a very poor blood-brain barrier. If you take out the trigeminal ganglion, it's blue, so it lacks the blood-brain barrier.

You can, of course, see that lots of dye mingle in the trigeminal ganglion. That would mean that this is an excellent site where your agents easily can go to. I think we have forgotten this kind of obvious place.

Another aspect is, you can see here, there are blood vessels. They're also full of Evans blue staining, so here is a good case that we have a lot of agents that can pass into that.

We have done some experiments using the MCA.

Am I out of time or are you getting tired of me?

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Just some obvious points. If we perfuse MCA, it maintains its blood-brain barrier. If we put in CGRP in this vessel, it doesn't dilate if we give it luminally. Again, it doesn't affect the endothelium, but if we give it abluminally, it dilates.

So I jumped on this. And if we don't take this same vessel -- here is the CGRP response. We put on CGRP abluminally, on the outside, where the nerves are. So this is sensory nerves going to the perivascular space. And if we put on a CGRP blocker -- this is the old one, CGRP 8 to 37. And if we put it abluminal on the same side, you can block the response. If you put it luminally, it has no effect.

Here is the case for the antibody. So if you put the antibody on the outside, you can block it. If you put it on the inside, nothing happens. And this is one of the gepants, same thing. And here is the NOX blocker. So it did, in my hands, very little even when it was put on the outside.

So that was for the trigeminal ganglion. I

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will just come to nearly the end here. The third place where we can imagine is the brain stem, the trigeminal nucleocomplex. And one thing which has been discussed is where do these trigeminal fibers go in the brain stem? Of course, to luminal 1 and 2, but they are separate.

So in the outer part, we see the fibers from the containing receptors, the A fibers, and on the slightly more inner part, that's where we have CGRP. And they are on separate fibers, so they could interact here in some way or, of course, to go to other parts.

I'm not boring you with any more of this, I think. The main issue, I would think, is that we should look more into the trigeminal ganglion as a possible site where we could have the therapeutic effect of the CGRP antagonistic agents. Thank you very much.

(Applause.)

DR. TURK: We're running a little late, and we want to maybe even end a little bit early. So what we're going to do is, I'll take two questions.

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We'll have a 15-minute coffee break. We'll come back for a last presentation and then a short panel. So two questions if there are. I know we're keeping you from coffee.

Allan?

DR. BASBAUM: Why not? What I've always been puzzled is -- not puzzled -- is there something unique about the trigeminal vasculature or even the ganglion? Because one tends to analogize to other parts of the body. And is it absolutely clear that the anti-CGRP has no effects in other parts of the body and in other conditions, even particularly, say, ischemic conditions where there might be a vascular component?

DR. EDVINSSON: One aspect with CGRP in the trigeminal ganglion, which is a bit unique, is that 50 percent of the neurons contain CGRP. So it's very high. And the other half contain the receptor, so it seems to be very concentrated on that. I haven't really gone through in detail the dorsal root ganglia yet, but it's a tempting scenario. Other signals, we have looked on in the

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trigeminal ganglion -- I mean, I have gone through a lot of them -- there is nothing standing out so beautifully as the CGRP system. So it could be that whatever, epigenetics or something, makes this kind of prime CGRP site.

When it comes to the ischemia question, there was one study on the heart. They had the dogs. They were exercised, and then nothing happened. But then if you make the coronary artery very much thinner, you can exercise them again, and they are on the verge of developing ischemia, but nothing happens. You give a CGRP blocker, same thing, but if you give it triptan, they develop an infarct.

DR. TURK: Any last question before we have a coffee break? And there will be a chance for questions a little bit later. So let's come back at 20 to -- what, Bob? Make it a short coffee break. Come back at 25 to 4:00.

(Whereupon, a brief recess was taken.)

DR. DWORKIN: So we're going to start again. Thank you all for your endurance. So we have one

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more presentation left and then we'll have a panel discussion, like we have had before, for about 45 minutes at most. And the plan is to call the meeting to an end at 4:45. So if you're planning to have a taxi or a shuttle bus outside at 5:00, you don't really have to rush and leave the meeting early because we'll end at 4:45.

So it's a great pleasure to introduce our last speaker, Dr. Marcelo Bigal, who was at Albert Einstein College of Medicine for a great many years. And then he moved a few years ago to Merck and, more recently, moved to Labrys, a small start-up in San Mateo, California. And so it's delightful to have him here with us to talk about clinical studies of anti-CGRP agents.

Presentation – Marcelo Bigal

DR. BIGAL: Good afternoon, everybody. I know it has been an incredibly busy two days for everybody. I'm going to try to be concise and make the clinical points here. As a disclosure, I am the chief medical officer of Labrys Biologics. Labrys is developing one antibody, anti-CGRP, and

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Labrys is in the process of being acquired by Teva Pharmaceuticals.

Before Labrys, I was at Merck, developing the CGRP antagonist platform. And I have been on the other side as well since 2001, heading two headache research centers in the United States, in Connecticut and in New York City.

What I would like to discuss with you is, I am not going to repeat what Dr. Edvinsson has presented already. He's the main source. He is the man that discovered and described the story. I'm going to, however, present my perspective on some specific aspects of the relevance of CGRP in migraine, then the challenges we face when we try to develop a small molecule for, again, CGRP, where we are in antibodies. And I'm going to talk a little bit about the one I am developing because it's one that I have more data about.

So CGRP migraine, migraine is a disease that actually is a disease of stages. The disease is artificially divided in chronic and episodic in absolute lack of data. Why? There is a stage of

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migraine that's called chronic and is defined when people have more than 15 days of headache per month and another one that is episodic, and they have less than 15. It's very scientific. It's like this, 15, less than 15, symmetric, symmetrical, let's divide the disease in two stages.

There is migraine. Migraine is a disease that may be very benign, and people have very infrequent headache attacks. And they treat with over-the-counters or symptomatic medication. Migraine may be a disease that effects individuals on a daily basis, and there is everything in between.

So it's a disease. And to complicate matters, meaning migraine patients evolve into a more chronic stage and remit to a more benign state through their lives until they find a stage where they belong and they stay there.

So I prefer to divide migraine as I am saying here, as single disease, with stages, each of them with different medical needs. So in the very low frequent stages, patients are well-

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attended with OTCs. Patients with more frequent headaches, we require the triptans, specific migraine medications that block the serotonin pathways.

These are easy to manage most of the time, easy-to-manage patients with low risk of complications, but as patients start evolving, some of them start developing more frequent forms of migraine. They require prophylaxis now, not only symptomatic medication. They require prophylaxis. And some of them, 1 percent in the population, will evolve to a stage where they have migraines on more days than not, full-blown migraines, headache, photophobia, phonophobia, nausea, the whole picture. And this can be incredibly debilitating, meaning incompatible with normal functioning.

The biology of the disease is the same, however, largely the same. There are added factors, risk factors, biological vulnerability, that stages may have that others don't, but the disease is largely once.

You learned this story about CGRP. Right?

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CGRP stands for calcitonin gene-related peptide. It's the most potent vasodilator that we are aware of. It causes brutal vasodilation when on the top of blood vessels. But more important, it was initially discovered when we were developing the triptans for the acute therapy of migraine.

We found that, actually, when blocking triptans, the therapeutic discovery, actually, Dr. Edvinsson discovered all these steps that actually preceded the triptans. But the therapeutic importance of CGRP became evidence when we realized that, when we give a triptan like sumatriptan for the acute therapy of migraine and we address the serotonin pathways, we would block the degranulation of the trigeminal nerve. And among the substances that were not being released any longer, we had CGRP.

At the time, we thought that migraine was a vascular disease, not as nowadays, that we know that migraine is a neurological disease. So basically, we were thinking about this vascular condition that I can give vasoconstrictive

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medications and block the attack. And by the way, I just discovered this important and potent vasodilatory molecule. Bingo. Let's block this to avoid the vasodilation because the vasodilation is the cause of the migraine pain.

It is not. Right? Although CGRP caused vasodilation, and although vasodilation caused edema in the blood vessels -- which blood vessels? The dura mater blood vessels and the blood vessels innervated by the trigeminal nerve. But actually, CGRP is indeed released when the trigeminal nucleus caudalis is activated.

So the vasodilation that happens in migraine is very late in the process of the migraine attack. And indeed, CGRP seems to be involved, but not only on vasodilation, but on an inflammatory cascade that happens outside of the blood-brain barrier at the level of the trigeminal endings.

But this is also not primary migraine. What's primary migraine is actually what you saw Dr. Edvinsson showing, some activations that happen at the brain stem. That's where migraine begins.

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Migraine begins when individuals that suffer from the disease, for reasons that we do not fully understand, have activation in the brain stem -- have activations in certain areas of the brain stem.

When they have these activations, the gate opens. The gate to what? To everything. Now, minor insults are perceived as severe pain. Normal light is perceived as photophobia. Normal sound is perceived as phonophobia. Right? Your brain became hypersensitive to the internal and external environment. Right?

So the gate opens and the gate is inside the brain, but the gate means nothing. It's like a road where the gate opens. The gate means nothing if a car doesn't go through. And what is happening, the CGRP, therefore, in my way of seeing, has a dual importance.

First, CGRP is represented here and is involved in the activations that actually are initiated in the beginning of the migraine attack and are involved in opening the gate. But CGRP is

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going to release it outside of the blood-brain barrier.

So inside, outside of the blood-brain barrier, creating a low degree of inflammation that's now perceived as a lot of pain just because the gate is open inside the brain. And this CGRP is going to cause this mass at the trigeminal endings. We also come and sensitize the trigeminal endings, causing primary sensitization, secondary sensitization, and eventually thalamic sensitization.

So it's released, and it comes back to bite the brain. It releases and comes back again. And patients will start having pain and photophobia, and phonophobia, and central sensitization. And therefore, they become more predisposed to pain and everything else. And they evolve from very low-frequency forms of migraine into very high frequency forms of migraine.

So you can block the CGRP pathway inside the brain. You can block the CGRP pathway outside of the brain. And, basically, as Dr. Edvinsson

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clearly demonstrated -- and the work was pivotal because it settled 15 years of discussion or more -- you can clearly have efficacy blocking CGRP with compounds that do not penetrate the brain.

So when we are giving these compounds, we are basically targeting the trigeminal ganglion and what else is distal to the trigeminal ganglia.

Very interesting, although CGRP is the most potent vasodilatory molecule that we know, blocking CGRP des not cause vasoconstriction. It restored the normal tone, as it does not cause vasoconstriction. I'm going to talk a little bit about clinical data on that.

Levels of CGRP are elevated. Peripheral levels and jugular levels of CGRP are elevated in the course of migraine attacks, as his pivotal work showed, and the levels are restored by administration of sumatriptan, as I mentioned.

The levels are further elevated in chronic migraine versus episodic. The more headaches they have, the higher is the level of CGRP, even in between attacks. I'm not talking during the pain;

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in between attacks. So CGRP is not only involved in the paraphysiology of migraine, it's involved in the paraphysiology of transformation from episodic into chronic.

So I told you a little bit about this story. We can block this molecule. We don't cause vasoconstriction. Let's go for it. It's much more selective. Remember, the acute therapy of migraine is started with ergotamine compounds, the specific therapy, that would block every single receptor that we know of.

Then we developed the triptans that are second generation or gotamines, where we block only certain serotonin receptors, and we stop the release of CGRP and other molecules. Let's be more selective. Let's try to block CGRP alone -- again only. I'm sorry, only.

Five small molecules have been advanced into -- this is just illustrative. Don't try to read this. But five molecules were advanced into phase 2, phase 3. They're small molecules. They never failed on efficacy. There has never been a

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small molecule that we brought into phase 2 and phase 3 that failed on efficacy. Nonetheless, we still don't have anything approved because of something that I'm going to discuss with you, that was a challenge, that forced us to change the paradigm.

The initial molecules were being developed for the acute therapy of migraine, not prophylaxis. Right? They were being developed to be used as a symptomatic. Patients would use when they have pain, only when they have pain.

The initial medication that was advanced into phase 2, Dr. Edvinsson mentioned, was olcegepant. Olcegepant was developed by Boehringer Ingelheim. It was an IV molecule when these studies were done. First proof of concept, beautiful. Right? I mean, classical pharmacology textbook, dose response, efficacy in the primary, efficacy in the secondary.

The efficacy was coming at a very interesting level. So for those of you that actually do not follow the regulatory path for

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migraine, the primary endpoint in acute therapies, for acute therapies, is a proportion of patients that become pain-free, zero pain at two hours. Two hours after administration, they become pain free. The secondary endpoint is called pain relief. They can have pain, but the pain has to be mild.

So for pain relief, two-thirds of the patients had pain relief; 42 percent had sustained pain free. This is a very strict endpoint. They become pain free at 2 hours and the pain does not come back in 24 hours.

All the medications that we have available in the market, the triptans have lower rates than that, no serious adverse events, no cardiovascular events. Remember, this is a vasodilatory. We started developing these. Our main and solo concern was hemodynamics. So nothing came here.

The medication was discontinued. And the reason given was that, actually, they could not formulate into just oral tablet.

The second medication was the one that I was involved in, and Dr. Edvinsson showed the data on

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the PET scan and stuff. It was telcagepant. Telcagepant was an oral medication. And here you have the two pivotals, Protocol 11 and Protocol 16 were the two phase 3 studies, clearly showing that, actually, the efficacy was aligned with a very good triptan, better than placebo for primary endpoints and secondary endpoints, but not only this, for photophobia, for phonophobia, and for nausea. It was an approved med based on efficacy parameters.

But something happened -- and save this, because there's something that happened with telcagepant that happened to most of the medications, as a small molecule. But the medication was coming clean, and beautiful, and most important -- and I really ask you to save this parameter -- placebo-like adverse events, every single CGRP that was ever developed has a tolerability that's similar to placebo, not telcagepant. All.

You do not find a pattern of adverse events. They are very well-tolerated. You don't find a syndrome that actually could -- so very tolerable.

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Patients receiving zolmitriptan, which is one triptan, 40 percent of them have drug-related adverse events versus 20 percent, 22 percent in the telcagepant.

As we were developing telcagepant, we were developing a back-up compound that was 10 times more potent than telcagepant. It was called MK3207. We didn't give a name because we had killed it before. We also advanced it into phase 2. It was an adaptive design, so it was an exploratory of the doses. So that's why you don't see a clear signal as you saw with the others, but that from an efficacy perspective, significantly more promising than telcagepant, which is the one that I just showed you, meaning this was coming really great, but something happened.

There were, so far, three CGRP antagonists and three affected stories. Boehringer, after olcegepant, developed a 4-CGRP receptor antagonist. And what they did here is, actually, they compared with an incredibly effective triptan called eletriptan. And what you have here is, actually,

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the efficacy of the molecule in early time points going all the way to 48 hours, and in very dark, you have eletriptan.

So another attribute -- I already told you about the brutal tolerability of CGRP antagonists. The other attribute of the compounds in general, the class, they are not the fastest medications in town -- triptans can be faster -- but they last long.

So if you start seeing -- I mean, they go in parallel to eletriptan. At two hours, eletriptan was better, at least numerically, than the doses of CGRP antagonists. At 24 hours, there is a reversal of the trend, at 48 hours a little bit, and it lasts. The efficacy of CGRP receptor antagonists lasts.

Everything that I am telling you here is for the acute therapy of migraine. And finally, later, Bristol-Myers -- and there is the co-author of this study over there in the back. Bristol-Myers developed a fifth CGRP receptor antagonist. The active comparator was sumatriptan. And once more,

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several of the doses came effective, and, once more, brutal tolerability. I happened to be invited to write an editorial to this paper that was published in Cephalagia a few months ago.

So the question is, actually, if they are always effective and they become tolerable, why don't they get into approval?

I cannot tell about the others, but I can tell you about what I did, the ones that I did. You have liver tox. So what happened with this CGRP receptor? You don't have liver tox when you give it sporadically. But because migrainers, many migrainers who have very frequent migraines, many migrainers will not use this medication sporadically. They may use it daily.

So the agency properly asks us to stress the molecule and create studies where we test the molecules in frequent paradigms of usage. And when we did this with telcagepant in a study that they were using daily -- as some people could eventually do in real life. But remember, 12 percent of the population has migraines. So a lot of things may

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happen and you have to stress the molecule.

What we've found is that, actually, the more frequent was the use, the more likely they were to develop hepatotoxicity. Nobody got really sick, but the increase was severe, as I'm going to show you. We did not know at the time that the accumulation of telcagepant was low creatinine [ph], so when we gave these very frequent exposure paradigms, we increased the area under the curve by eightfold, so we largely gave a lot, more than we should.

But we saw this pattern in other studies with other molecules, where we were giving less. And the reason was never related to the CGRP, meaning knock-out animals have no liver problems. With the antibodies that you are going to see, there is no liver problems. The problem was with the molecule and the fact that we rescaffolded the same molecule into its likely different copies, trying to improve the qualities of the molecule. And we were scaffolding the liver tox group in the molecule.

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The liver tox could be like this, meaning look what happened with the ALTs in some patients. Very scary. So we discontinued telcagepant. The rate of liver tox with telcagepant was lower than what we have with a proven medication such as Maxoud, significantly lower than what we have with Tylenol, only happen at frequent exposures, but it eventually caused a voluntary discontinuation of the program. The FDA didn't even wait on this.

So then, after five trials and five failures, what's next for us? There is something here. The medication works. The tolerability is excellent. So when you have medication, so for migraine, every time you have something that's very tolerable, it becomes a great candidate for prophylaxis.

Efficacy drives the Q2s [ph]. If you are miserable because of pain, you are going to take whatever they give you, and you are going to accept the side effects. But if I'm asking you to use something for months in a row or for years in a row, it better be tolerable.

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So what we tried to do here is actually switch the paradigm and use the fact -- take advantage of the fact that monoclonal antibodies are not created in the liver, are very unlikely related to liver toxicity. And however, they have incredibly long half-lives.

So what can I do if a medication that has a very long half-life, is very tolerable for my prophylaxis? I can give very infrequent use of something for patients that are currently absolutely miserable with what they have, mainly patients with chronic migraine.

I'm not going to discuss this with you, the entire course, for there are many talks about this, many. But we took advantage of the exquisite specificity that antibodies have. They go to CGRP and only through CGRP, nothing else.

They do not go to any of the cousin molecules like amylin, adrenalmedullin, calcitonin. The half-life that they carry is, if they prove to be safe, an incredible advantage, meaning you can get these patients -- so for example, the

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medication that I'm developing has a 45-day half-life. So you can basically, if it works and if it proves to be safe, actually take these patients that actually now have daily headaches, daily migraine headaches, and use two or three classes of medication twice of them each, and eventually treat with one injection every six weeks or an injection every month.

You know how monoclonal antibodies are developed. I am not going to spend your time here. You're all bored. You're all tired. But actually, it was a paradigm shift. It's the first time that we are developing a biological for the treatment of migraine and headaches. Biologicals are known for a neurology. Biologicals are known for pain, but never for migraine.

So all the processes that other areas acquired very early in the development, such as master-cell banks and all the quality control, is new to the migraine field, and I think we are doing well.

The monoclonal antibodies that are being

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developed are either fully humanized or human. And I'm going to talk a little bit about this, meaning there are several -- meaning, of course, developing a monoclonal antibody is very different than developing a small molecule, and the studies are different, and the paradigm is different. And there are four companies navigating this ocean, and I'm going to share a little bit of this with you.

I'm running a little bit on these topics that I assume you guys are very comfortable with, but I'll be happy to discuss in the Q&A any details that you have.

Right now, there are four antibodies being developed against CGRP. The one that I am developing is this one. And I'm going to talk about what I know about the other three, what's in the public domain, and focus a little bit on us.

So one important difference is, actually, one of these antibodies, the antibody being developed by Amgen, targets the receptor to CGRP. The other three target the ligand CGRP receptor. Three of them are going for episodic migraine,

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which is actually high-frequency episodic, patients with less than 15 days of migraine that require prophylaxis. And we are going for both. I find it of absolutely no sense to treat people with 5 to 14 and not 15 to 30. So we have an individual development, one trial for episodic, one trial for chronic.

We are all in phase 2. All of us are in phase 2. Most of us are subcutaneous. One is IV. They are developing a subcutaneous. And the paradigms of that administration vary according to the half-life of the molecule.

So let's talk a little bit about the clinical. You asked me to come here and talk about what's available clinically. I did from my small molecule perspective. There is still very little on clinical efficacy for the antibodies, short of the company's release of data in the form of abstracts. So everything that I am aware is in this slide.

The first one is this medication calls ALD-334. This medication, different than all the

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others, is not manufactured using a mammal cell platform. This is yeast, and there are pros and cons. However, they validated the target. Basically, it's very interesting. They gave a very simple phase 2 study, one IV dose, very high dose, IV, once. Why? Because they did not yet have the toxicology package required by the FDA.

But although they only gave once, they followed them for six months after a single administration, not monthly administration. And as you can see, with a single administration, 67 percent of the patients had very meaningful improvement over the first three months of the study, a single administration.

This was superior to placebo, although this is the highest placebo rate I've seen in a migraine trial in ages. And we can discuss a little bit about this in a migraine trial.

So the therapeutic gain is not that impressive, but if you consider that they only gave once -- and this is a competitor, so I have no interest in coming here and -- but we have to give

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credit where it belongs. If you only consider that they gave only once and they were able to find superiority statistically, that's something to pay attention to. They do not penetrate the brain, which validates what Dr. Edvinsson said. We are going to the trigeminal ganglia and to whatever comes downstream to that.

The second antibody that released data is being manufactured and developed by Lilly. They had the toxicology package in place before they started phase 2, so instead of doing what the first one did, they gave subcutaneous administrations every two weeks for three months. It resembled what phase 3 would look like for them, double-blind placebo-controlled, and very similar results, 63 percent efficacy, very high placebo rate as well.

Both of them included patients with less than 15 days of headache per month. So this is the efficacy for the antibodies available so far. There have been five small molecules. The five were effective, so there are four antibodies. Two

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have efficacy data so far in early stages. Two are more advanced in Amgen, but still do not have efficacy data where it should be. And so far, the two antibodies succeeded.

This is us. LBR-101 is fully humanized at IgG2. We engineered two mutations to avoid effector functions. We on purpose want to stay away from the receptor. And the reason being is that we don't know who is right. There is one company that has an incredible know-how engine that is going to the receptor, and there are others that are not.

My justification is, I don't want to knock out the signaling. I don't want you to bring the signaling down to zero. This is not acute therapy. I want to have a pool of circulating CGRP able to be recruited to homeostatic functions. You don't have a molecule that's so distributed doing nothing.

So we want to preserve some tone [indiscernible]. That's why we are not going to the receptor. Actually, we voluntarily induce

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the 2-point mutations to stay away from the receptor. We don't bind to anything else. We only bind to CGRP.

We concluded six phase 1 studies already, five with IV, going as high as 2,000 mgs. The highest dose we were testing in phase 2 is 900, in phase 2. But in phase 1, we came all the way to 2,000 mgs. And in the toxicology package, which I didn't present since I was asked to focus on clinical -- but in the toxicology package, we went to 300 per kilo per week, almost as much as the weight of the animals.

We did six phase 1 studies to bridge the IV formulation to subcutaneous formulation. The half-life of the molecule varies a little bit according to the dose we are giving, but it's around 45 days. And we are in the midst of two phase 2B studies, one for chronic migraine and one for high-frequency episodic migraine.

In each of these three studies, we tested two doses versus placebo. And the doses are different and staggered. So in between the two

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studies, we are testing four dosing paradigms. There is no reason to believe that something that works in chronic does not work in episodic and something that works in episodic does not work in chronic. It's irrational.

So we are taking is advantage of these and testing for those new paradigms. They are both underway. Both of them have -- altogether, we already have around 280 patients in phase 2. Several of them already finished. They are going to receive medication monthly for three months. So the duration of this study is four months. These studies are parallel. There are three doses, three dosing, and four dosing paradigms.

I'm blinded. I don't know if this is going to work or it's not going to work. But I tell you that, actually, we only lost two patients after randomization, so only two drop-outs, none for adverse events. It's an attribute of the class.

Everyone else is going to come like that. It's an attribute of the class. It's even difficult for us when I was writing the

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investigator brochure that, as you know, you have to release what are the expected adverse events. I had trouble coming with the adverse events because we see very little.

If phase 2 becomes positive, we are eventually going to start a dual phase 3 program, one for chronic migraine, one for high-frequency episodic migraines. This is just details of the IV-completed studies. Many doses that we tested in IV were super therapeutic. I don't know about super therapeutic, but actually significantly superior to the doses that we are testing in phase 2.

This is phase 1. Treatment-related adverse events happen in 17.8 percent of participants receiving placebo, 20, 22 percent of patients receiving LBR-101. The rate did not increase with the dose. So 2,000 mgs had the same rate of 1,000 mgs, which had the same rate of 250 mgs. There was not a dose dependent, and we see this again, and again, and again.

I have one final slide, and I want to

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preserve time for the discussion, but I just want to pause a little bit here and say the main concern -- and I've been doing this now for almost a decade. The main concern of inhibiting CGRP was what happens to your body when you inhibit such a potent vasodilator.

So in the very beginning, we were insane about that. Two knock-out models of mice were created without any CGRP. They do not have increased blood pressure. They are not more likely to die from cardiovascular events. Actually, they live longer. Go figure.

When we were developing telcagepant, we got to the extremes of the study that Dr. Edvinsson mentioned. So we did pre-clinical studies in pigs, in dogs, in human coronaries, for clinical animal studies. Then we got to a point where we did one study for telcagepant in humans with stable angina.

So we basically got patients with stable angina, ran them, of course, in an intensive care unit and, of course, upon the request of the agency.

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We randomized the patients to a treadmill test until pain. So they were randomized to receive placebo or four times the highest dose of telcagepant, four times the highest dose that we were testing in clinic. They walk it until pain, and there was no difference.

So now, we build so much confidence that, actually, this is less of an issue, that we are moving to other issues. What about infusion reaction? What about potential for immunogenicity or for things like that? But obviously, in phase 1, we were -- I am sure the others were as well. I have hours.

We were almost insanely looking for cardiovascular signals. So everybody in our phase 1 study stayed confined for seven days. The Tmax and the IV was three hours. For example, just inn day 1, where the Tmax happened, they would be on continuous monitorization plus 12 EKGs. Then we did triplicate EKGs daily for seven days. Then we followed these people that receive medication only once for 90 days. And we just published these

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results and nothing happened.
Did it surprise us? No, it didn't, because actually, now, at this point, I think we are getting to a point that we totally understand that there is so much redundancy in the homeostatic control of cardiovascular functions. My understanding now is CGRP over evolution -- CGRP is so preserved. And it started in evolutionary terms as a cardiovascular molecule and evolve into a neuropeptide that is key in pain transmission and pain modulation.
So in summary, CGRP is a relevant molecule for a migraine. CGRP receptors, antagonists have never failed on efficacy. CGRP antagonists have been difficult to develop due to issues of liver toxicity.
Monoclonal antibodies may target CGRP, as we are doing, or its receptor, as Amgen is doing. They are not degraded by the liver. And several antibodies, anti-CGRP, are currently in development. Thanks for your attention.
(Applause.)

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DR. DWORKIN: I'd like to invite Drs. Rice, Katz, Edvinsson, and also Dr. Rappaport, who is director of the Division of Anesthesia, Analgesia, and Addiction Products at the FDA to the panel. And what we'll do is -- and you can all have a seat up here. And I will keep my promise of ending in exactly 30 minutes. And so let's start with any questions for Dr. Bigal since I preempted a discussion section for his talk. Steve?
DR. MCMAHON: So one question on the CGRPs. It may sound slightly esoteric. About five or six years ago, there was a reasonably high-profile paper from Jeff Mogil, who is a geneticist, and Peter Reeh, who is a pain electrophysiologist.
The thrust of this paper is that it started with Jeff Mogil's interest as to why different mouse strains have different pain sensitivities. He did the genetics and Peter did the recordings and the functional studies. And the conclusion of the paper was, the mice strains have different pain sensitivities because they release more or less CGRP from peripheral nerve terminals

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constitutively. And that was supposed to act back on auto receptors.
I don't know if that paper was true, but you have a great opportunity to test some of those ideas in humans. So a direct prediction of that paper would be that CGRP blockage would lead to an increase in heat pain threshold. People will become less sensitive.
It's a very simple measure to do that's done every day in all kinds of testing. And I wondered whether you've ever looked at that or seriously considered the possibility that the mechanism they propose might be a side effect in your treatments. And if it's true, it could actually be of relevance to an understanding of the pathophysiology of migraine.
DR. BIGAL: Thank you. I appreciate your comment. It's unfortunate that Dr. Brick [ph] is not here anymore. He is doing a study using our antibody, where he is basically inducing allodynia into rats. And he is using two models. One model is, he induces allodynia with sumatriptan. The

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other model, he induces allodynia with opioids. Some of the animals are pretreated with placebo. Some of the animals are pretreated with our antibody.
These are not migraine models. These are pain models. And he demonstrated what you said, when you knock out CGRP in the animals, in both allodynia models, the threshold -- it's not a hyperalgesia model. It's an allodynia model, but it goes to the same direction that you predicted.
DR. MCMAHON: That begs the question [inaudible – off mic.]
DR. BIGAL: I think it's relevant to migraine progression. So basically, when people evolve from episodic migraine into chronic migraine, the biological features of migraine are retained, but migraine acquires components of chronic pain now. So episodic migraine is pure migraine. It has very little resemblance with chronic pain syndromes.
Chronic migraine is migraine plus chronic pain. They acquire everything else. They acquire

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the allodynia. They require the extracephalic allodynia. And they acquired all the co-morbidities of chronic pain. So I think that's why I'm so keen to study these on chronic migraine because I think that, actually, this molecule, based on what we did at Merck, has the attributes to revert the biology of migraine and the secondary chronic pain features that develop over the years.

DR. MCMAHON: I don't want to keep going on about it, but they have an interesting mechanism. They claimed that the animals that release lots of CGRP acted on autoreceptors to further reinforce CGRP production. So actually, a migraine is, you could imagine, a slow cumulative wind-up of the whole system that would take you from normal, to occasional, to a kind of chronic migraine stage if it was true. Yes.

DR. BACKONJA: Actually, just a follow-up on the same question regarding the sensitivity -- I'm aware that some of the investigators have done quite the sensory testing in patients with migraines and it clearly demonstrated the presence

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of allodynia and hyperalgesia as a manifestation of migraine.

Are you aware of or do you have any results of the effects of any intervention of either small molecule or antibody on that sensitivity, that a change improved, got worse?

DR. BIGAL: I am. So allodynia, meaning over the past years, trigeminal allodynia, is the most potent marker of migrant refractoriness in clinical trials. So basically, when we were developing this, the reason I ask -- we worked with Brick on the allodynia models is because we strongly believe that, if you do not revert to allodynia, you may symptomatically treat migraine, but you don't bring them back from chronic to episodic. Allodynia is key here as a marker of trigeminal sensitization.

So we developed and validated a questionnaire called the ASC12, the Allodynia Symptom Checklist 12, versus QST for using migraine and migraine research. So we are talking about trigeminal allodynia only. And our phase 2

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clinical trial applies there. Everybody is having their allodynia captured by the questionnaire.

So one of the a priori described analyses that we are going to do is actually the efficacy as a function of allodynia or not.

DR. BACKONJA: But nobody did any of the quantitative sensory testing like Rami Burstein or a few other investigators. I mean, I am not aware.

DR. BIJAL: We did not. So Rami Burstein allodynia studies for us with animals. So we developed one CGRP antagonist called AA25. And he used his animals of inflammatory soup to induce allodynia in animals and show that, actually, triptans could not revert several of these neuronal activations in the presence of allodynia, but CGRP receptor antagonists did.

What we did not do and we are doing now with our trial -- but what we did not do is in humans with QST.

DR. DWORKIN: Other questions for Dr. Bigal?

(No response.)

DR. DWORKIN: I don't want any of you to

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leave this afternoon without having had your questions answered for this panel. So any questions from the audience for any of the other speakers from this afternoon's session?

(No response.)

Q&A and Panel Discussion

DR. DWORKIN: Then I will ask the members of the panel. So the focus of this afternoon's session was translational success stories. What Allan, and Dennis, and Frank, and I hoped to put together here were three examples of the success of translation from pre-clinical to at least one, and often many more, demonstrations of efficacy in clinical studies. And so we've seen three examples of that.

I'd just like to ask the panel to comment, are there any kind of take-home messages from these three translational success stories? Can we conclude something about the characteristics of successful translation from pre-clinical to clinical? Andrew?

DR. RICE: Can I start with what I hope will

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be a slightly provocative comment, but could be something that the group actually could take forward?

I was in conversation with my colleague the other day, and he made a quip that I thought was very true. To translate, you need to speak two languages, at least. And one of the things that we tend to do is work in our silos of pre-clinical and clinical. And the clinical people have a lot to learn from the pre-clinical people and vice versa.

The past few weeks, I've been thinking about how we could facilitate that. One way might be to encourage internships of trainees in research, so that basic scientists might come, and spend some time doing clinical trials, and seeing what the issues were, and vice versa because I do think we tend to sit -- we assume that we know what the pre-clinical people do.

We take their word for it and vice versa, and they seem to believe that we know what we're doing in clinical trials. And a little bit more learning of each other's languages, I think

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wouldn't be a bad thing. And that's exactly something that a group like ACTTION could probably facilitate, or at least we could talk about how we would facilitate it. It is actually what we're doing here already.

DR. DWORKIN: But you're saying something a little bit more intensive, that there would actually be a period of time that the individual would spend in a lab of someone doing something different than what they do. Potentially, ACTTION could support something like that?

DR. RICE: I don't know whether anybody's got any comments on that, or is it naive?

DR. BIGAL: I think there are two translations that need to be done, and one translation is this translation that we discuss here today. So for example, if we take the example of the CGRP drug development, it represents the accumulation of knowledge of many largely driven by Lars.

If he once was not doing the trigeminal vascular test, and describing the trigeminal

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vascular loop, and showing that CGRP, and not substance P, and not VIP, or the ones released, and map this into the biology of migraine, we could never develop.

I think that, actually, the age of serendipity is over, and we do not know how to develop drugs differently anymore. We need this translation.

The second translation that I think is important is, when you go to this science, translational science, as this requires, we very often miss a second translation that's needed, which is basically, science apart, what patients really need.

Now, we know the science. I know that I want to go to the trigeminal ganglia. I develop a molecule that goes there. But when it comes to develop the clinical trial, who are these patients? In real life, a patient with chronic migraine who would use something as I am developing, would they stop the medications that they currently are on before starting or phase out before starting? Or

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would they add this medication on whatever they have? If they improve, they phase out, meaning, who are these patients? Are these patients, patients that are going to use generic first? And once they fail, they would go to a high-end therapy like that?

This translation, I think, is where we are missing, trying to identify now that we know the signs and that we are developing the clinical trial, who is the true beneficiary of something like that, and develop a clinical trial that reflects the population?

DR. DWORKIN: So let me follow up. Are you suggesting that the reason for the success of the CGRP antagonist studies -- and you said all of those trials were positive, and it looks like the data we have from the antibody studies also, so far, are positive -- are you suggesting that the reason that those trials were all positive has to do with really thoughtful selection of the patients that were enrolled in the trials?

DR. BIGAL: No. The reason I think they are

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coming so nice is because the translation from basic to clinical has been well done. I think that's the reason. I think the second step, the future step, is now how to bring this a step forward. And I don't mean cherry-pick the population, but basically really try to tailor the clinical development and make the clinical studies and the clinical trials reflect the population that actually is going to end-use it, because right now, my point is, 12 percent of the population has migraine.

About 12 percent of them will need an antibody. Who are they? How do they look like? What are the co-morbidities that they have? And instead of excluding these guys, as we typically do, go for them.

DR. DWORKIN: Maybe we should.

DR. BACKONJA: I would just go back to a more general comment that Andrew made. And I will definitely say that we are in terrible need of really better communication, again, because we definitely do speak two different languages, basic

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science language and a clinical language.

For this to be a translational endeavor, we really have to speak the same language and probably a little more disciplined the way we use our language because, in pre-clinical settings, we do not study pain. You study nociception and neuropathic pain processes. But when do they become pain is -- human subjects say they have a pain. Otherwise, before that, it's all nociception.

But it goes back to -- in some of the words used during this meeting, I have to give credit to Allan, who is gone. He made it, in his presentation, clear that he was studying mechanical hyperalgesia or thermal allodynia. And that's what he staffed, rather than generalizing it to pain in general.

I think that these are the kind of steps that we need to do. But do we really need to go back to where and how do we do this? There's really no good model and maybe an occasion like this is a good model just to really start to set

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the criteria for good translational pain research.

DR. DWORKIN: Dr. Edvinsson?

DR. EDVINSSON: I think you touch a very important issue. I think, for my part, I am a clinician also, and I have a good laboratory. And that makes it more easy to see the patients' needs and the way to translate it. But I think it would have been difficult for someone just training as an M.D. to see how the lab works.

I did a long period in my young days to do my PhD in a neuroscience laboratory, so then I can understand the methodology. And I think you need to have this kind of MD, PhD training to understand the methodology that the great guys in the novel technologies have. So you must share, get it together.

DR. RICE: I just wanted to completely concur with you and Misha and ask you, Misha, one of the phrases I heard today was -- or maybe it was yesterday -- measuring symptoms in rodents. And to a clinician, that's anathema. You can't measure symptoms. It's something that can't communicate

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with you.

But I think, in neuropathic pain, I've said it many times, we suffer from a rather bizarre disconnect. But by and large, we tend to do our pre-clinical work in models of traumatic nerve injury, and our outcome measures are, at best, something we use to profile patients in clinical trials. That's certainly not an outcome measure, in other words, sensory thresholds.

Yet, in the clinical trials, we'll often do a product predominantly in polyneuropathy or PHN and measure pain as an outcome. There's just a really obvious disconnect there that, sometimes, we don't talk too much about. And I don't want to repeat last year's meeting, but there's also the whole issue of design standards by its reduction methodologies in pre-clinical research, which is a whole other meeting, but they merit just mentioning.

DR. DWORKIN: So Andrew, I have to ask this question. As I understood your comments, they assume that there's a kind of history of failed

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translation, of compounds that were efficacious in pre-clinical models that didn't show efficacy in the clinical situation. And there is such a large history of failed compounds. I mean, we all talk about NK1 antagonists. But I just don't know of that many clear examples of a compound that showed maybe not even a robust pre-clinical pattern, that when done in careful clinical studies, failed to show efficacy.

So there's a premise I'm not sure we have solid data for, that there are a lot of failures of the pre-clinical models.

DR. RICE: We are doing some work on that. But I think publication bias, both at the clinical trials that failed -- which is probably less than we think it is. We recently have not published it yet, but you were at the meeting where we had an assessment of publication bias for clinical trials. It seemed to be surprisingly low compared to the pre-clinical studies, where it's much higher.

So it's actually gaining access to the evidence. And one thing that surprised me recently

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is, we are currently conducting a meta-analysis of all pre-clinical neuropathic pain studies that involved behavior and outcomes with a pharmacological intervention in animals. The first sweep of that revealed 65,000 publications. When we've got those down, we're now somewhere around 6,000 publications that are admissible to a meta-analysis.

You compare that to roughly 220 randomized control trials that will be in the latest meta-analysis of neuropathic pain. We're dealing with a totally different scale. But the problem is we don't have a meta-analysis yet -- we are conducting it -- of the pre-clinical data.

Once we have that, probably later this year, I think we'll be in a very different position to be able to address that issue.

DR. DWORKIN: Steve?

DR. MCMAHON: So we heard on your part -- we heard from Chas yesterday that his numerical assessment was, what, 90 percent of compounds failed in phase 2 trials? And that's for

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lack of efficacy. And actually, implicit in your last comment was that was all due to the pre-- clinical sides. But don't you get that it was also due to poor clinical design. Even with the NK1, we said, for antagonists, astonishingly, when the knock-outs were evaluated, which they were being done whilst the clinical trials were going on for the small molecules, the knock-outs had very little phenotype other than in a visceral pain context. And as far as I know, that's never been tested clinically.

So you could even argue that good old NK1 hasn't even been disproven as a target. But my main point was that maybe at the end of two days, we should be smug and slap ourselves on the back, three examples sitting there of positive efficacy in phase 2 in the last few years. Another one supposedly announced last week, at least in the form of a press release from Convergance, who claimed that they have positive data in trigeminal neuralgia with their sodium channel program, and another one last year and being followed up on P2X3

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receptor antagonism. So that's not 90 percent failure.

Maybe we're doing something wrong in the sense that we have got -- definitely, there's been a lot of navel-gazing and hard thought about the animal models, the measures we have. There's a wider range of animal models being applied. I think the tests are being applied more rigorously. We have heard a lot about how clinical trial design may have been improved.

So maybe this is the beginning of a golden era where I am starting to see a lot of successes.

DR. DWORKIN: So Steve, I completely agree with you. And I want to highlight something that Andrew said earlier about an as-yet-unpublished study of oxcarbazepine in patients with, I guess, a variety of peripheral neuropathic pain conditions.

So in fact, there are, in the literature, three negative trials, phase 3 negative trials of oxcarbazepine and diabetic peripheral neuropathy, and I think one positive trial. And so that drug never made it to the clinic because of the three

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negative trials and one positive trial.
But now, we hear that in a trial conducted carefully in Europe with heterogeneous patients, not just diabetic neuropathy or PHN, and sophisticated quantitative sensory testing, that they were able to support their prediction that the drug was efficacious in patients who had, what, pinpricked hyperalgesia. Right? Something like that?
DR. RICE: It's not my study, and I can't give too much away because it's under review. Basically, it was the German Neuropathic Pain Network full protocol, and the Germans have developed and published a system for assigning sensory loss or gain as a phenotype.
DR. DWORKIN: To me, that raises the question of whether the three large, very large, negative trials of oxcarbazepine, a sodium channel blocker, of course, in patients with diabetic neuropathy were falsely negative; and that when it's done exquisitely carefully by a group of European academic investigators, this

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anti-epileptic shows efficacy in the patients we would have predicted it would show efficacy in. Nat, you have to say something.
DR. KATZ: I am going to say something, but probably not what you think I'm going to say.
(Laughter.)
DR. DWORKIN: I actually have no idea what you're going to say.
DR. KATZ: I'm yet again going to wander into dangerous territory, which is to talk about the pre-clinical data. And I want to pick up on a point that you just made.
I've got this idea in my head that the pre-clinical models fail to predict human efficacy. And the reason I have that in my head is because I have done dozens of studies over the last 15 or 20 years of new molecular entities that were presented to me as years of pre-clinical data, we're taking this into humans. Can you help us design a study or carry out a study? And we do it and it fails.
So that's happened to me numerous times, and

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most of the clinical studies never get published for all the reasons that we're all familiar with.
But now, as I think about it, I never really had a sense of responsibility to look at the pre-clinical data myself and to ask myself the question, "Do I really feel like these data are robust enough to take into humans?" because I didn't feel I had the qualifications to make a decision like that.
But now, as I think back upon it, it seems to me that, rarely was it the case that the pre-clinical data met any of their criteria that I put on my slides earlier. Rarely was their replication in multiple laboratories. Rarely could you find out whether the pre-clinical animals were randomized, whether it was blinded, et cetera, et cetera.
I really have no idea whether the pre-clinical data that I saw was robust data or not robust data. This whole connection between, oh, the pre-clinical studies showed this and the clinical studies showed that, I don't know what

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those pre-clinical studies showed.
So lately, in the last year or so, as I become more aware and sensitized to the flimsy nature of a lot of the pre-clinical data that comes to me, I've gotten into the habit of asking people who come to me now, and when I see the usual slides, "Just out of curiosity, was that study randomized?"
I never can get an answer to that question. "Well, just out of curiosity, was that study blinded?" "I don't know. I guess I'll have to go back to the people that did it." "How about any other labs? Did any other labs replicate that study?" "I don't know. Maybe I ought to go back." And these are people who are controlling millions and millions of dollars of investor money.
So I think, in a way, we have to go back and ask ourselves, what did those pre-clinical studies really show? And maybe your meta-analysis will get us there, but I think, based on what's published in the literature, with the enormous publication bias that we all acknowledge to exist, I'm not sure how

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to figure that out.
DR. RICE: I mean, I think there's one probable truism, that most of the strategies and models we have are good at predicting the drugs we know work. They're not good at predicting the drugs that would fail. And I think that's an important message.
But you wouldn't be surprised to hear I totally concur with you about the reporting of methodological quality. And we can take a lot from my collaborators in the stroke field, Malcolm MacLeod and his group, in Edinburgh, who have done a lot of work on this.
We certainly know that classic things like randomization and/or studies that fail to report -- that's all we can say. We can't say whether studies did it or not. But studies that fail to report blinding and randomization tend to overestimate efficacy, as you'd expect.
But about 30 percent of reports in neuropathic pain models report blinding, so we can take some encouragement from that.

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I think there are other facets of that, that are virtually never reported, and that is sample size calculations and, even more importantly, the predetermined criteria for excluding animals from the analysis and if animals were excluded from the analysis.
From the inquiries we made, that is actually quite a widespread practice to take out animals, and we had an ACTTION meeting on this topic last year. Animals are often excluded from the analysis without being declared in the write-up, and that's a cultural issue. And that seems to be a major bias that has not been addressed.
This area has moved forward fantastically in the last two years. We now have something called the ARRIVE guidelines, which is standard reporting guidelines, rather like CONSORT, which have been adopted by over 350 journals now, by certainly major U.K. funding agencies. The NIH have looked at it and proposed similar type-reporting standards.
So I think we are going to move into an area

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where we will have better reporting standards. And we remember what difference CONSORT made to clinical trial methodology. And I'm sure that will have some impact.
So again, I share Nat's optimism that there's no point in looking too much into the past. There's a lot that is changing, and they are examples of success. But again, I think it speaks to learning to speak each other's languages. Some of what we do is obvious to us in designing clinical trials and not necessarily obvious to a clinical trialist in animals.
DR. DWORKIN: So we're coming to the end, and I just want to give Dr. Rappaport a moment, if he'd like it, to comment on the presentations we've had this afternoon, the successful translation from pre-clinical studies into phase 2 and, ideally, phase 3.
DR. RAPPAPORT: I think I'll use the opportunity to comment more broadly on the meeting both days. I hope that we are in the beginning of a golden age. That would be wonderful. It needs

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to move forward more quickly. And I am obviously a big proponent of public/private partnerships, so I was really quite impressed with what Chas Bountra has been able to do in that paradigm.
I think we need to move further in that direction in the pain area because what we can accomplish working together in the private and public sectors, in the pre-competitive area, to share with the general community, that can then be picked up when we have evidence of efficacy or preliminary safety data, whatever it is that we need to do, and then share it, and then at that point, have industry pick-up and develop a product, I think we could move things so much more quickly.
I think, with the advent of better methodology and reporting in the pre-clinical area, I think that will really speed things up, too. So I think there are all kinds of things coming together right now, and the best way to take advantage of this is to -- I mean, I have obviously somewhat of a personal stake in ACTTION, at least for a while, and I think that this is the right

place to center what we're doing in pain because we have representatives from all of the important sectors, including the advocacy groups who are represented here today. So I just want to put in a word for pushing that direction.

If you see a way to share what we're doing, I think that's what we should be doing.

Adjournment

DR. DWORKIN: Thank you very much. I think that's the perfect way to end these two days. Thank you all very much for your participation, for your endurance. And if you want to get in touch with us, e-mail us, go to the ACTTION website for updates. And if you'd like to be on the distribution list for ACTTION newsletters, just let me know.

Thank you all very much and safe travels.

(Whereupon, at 4:44 p.m., the workshop was adjourned.)

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