



## Futures in Biotech, 47: Genetic Engineering in the 21<sup>st</sup> Century

### Leo Laporte

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[Music]

### Marc Pelletier

Welcome to Futures in Biotech. I am Marc Pelletier. Today we have a brilliant guest, and to help me with the interview I've invited Dr. Andre Nantel. He is a very good friend and one of the most enthusiastic molecular biologists that I know. So I've invited him to help me out here with the discussion. He is a Senior Research Officer at the National Research Council of Canada and Adjunct Professor in the Department of Anatomy and Cell Biology at McGill University, Montreal. Welcome, Andre.

### Dr. Andre Nantel

Hi, Marc, it's a pleasure to be here.

### Dr. Oliver Smithies

Hi, Andre.

### Dr. Andre Nantel

Greetings, Dr. Smithies.

### Dr. Oliver Smithies

Call me Oliver, simpler.

### Marc Pelletier

That's the voice of...

### Dr. Oliver Smithies

That's what I'm always known as in the lab.

### Marc Pelletier

Great.

### Dr. Oliver Smithies

You can call me Oliver with pleasure.

### Marc Pelletier

So that is the voice of Dr. Oliver Smithies. He is a 2007 Nobel Laureate in Physiology or Medicine for his discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells. He is also a professor in the Department of Pathology and Laboratory Medicine at the University of North Carolina in Chapel Hill. Welcome to the show, Oliver.

### Dr. Oliver Smithies

I'm glad to be with you.

**Marc Pelletier**

It's a huge privilege for us here to join your lab. This is the great thing about Skype. It gets us into the lab and it's fun to see all the great equipment in the back. I'm trying to scope out you know, what you use, what are your methods.

**Dr. Oliver Smithies**

Yeah, it's a messy place. My bench is not known for its tidiness.

**Marc Pelletier**

That's okay. Since our last show and I've observed your bench – my bench is now the same way. So there is going to be a lot of people emulating you for this. Andre was saying to me before the show that he was – he did his Ph.D. at the University of North Carolina in Chapel Hill. Andre?

**Dr. Andre Nantel**

Yup. Actually just across the parking lot from where you work. I was in Coker Hall.

**Dr. Oliver Smithies**

Oh, very good. I don't think we ever met when you were there though.

**Dr. Andre Nantel**

Actually there's a strange correlation between when we were there and what happened with your lab is that we had some very good friends. They are still there actually. One of them is Christian Jobin, who is working close to where you are. And they had friends with – it's either your labs or with your wife's because they were described as one of the leading labs in producing mouse knockouts. And they essentially gave us a corn snake, which was the first snake we ever had, and we've essentially had reptiles ever since.

**Dr. Oliver Smithies**

Oh, very good.

**Dr. Andre Nantel**

So it was kind of interesting when Marc was telling me where you were and I've Googled your name and we're like – oh, that might be – that's an interesting correlation, but we were there between 1991 and 1995. So we got to see the Tar Heels win the basketball championship and my youngest daughter was born there. I got the bill, decided to go back to Canada.

**Dr. Oliver Smithies**

I spent quite a bit of time in Canada because my first job was in Toronto, University of Toronto, Connaught medical research lab. I was there for about seven years, I think.

**Marc Pelletier**

Andre, you were mentioning the snake though, but what was interesting about what the snake ate.

**Dr. Andre Nantel**

Well, it was essentially being fed on heterozygote transgenic mice. So there was – and like any good mouse lab there's a lot of, let's call it, I shouldn't say that, wastage.

**Dr. Oliver Smithies**

Byproducts.

**Dr. Andre Nantel**

So it was so used to eating transgenic mice that once we switched him to pet shop mice, and people don't know that most of the mice in pet shops, they're not there for pets, they're there as food. And it took him a while to get used to pet shop mice. He didn't eat for like three months.

Now he was fine. We had him for like eight or nine years before he died of old age and we've moved on to water snakes, but it was always kind of interesting.

### **Marc Pelletier**

On knockout mice? It's just a – it's a cool correlation there that your snake was very fortunate to eat perhaps some mice that led to a Nobel Prize, some of your experiments that had double benefit.

[5:52] So let's get on to the show. I really want to ask Dr. Smithies like in the past show, in part one of this two-part series, we talked a little bit about the history of genetic engineering with mammalian cells. We talked about recombinational cloning, getting a gene into a mammalian cell line and we talked about how you came about to have the idea to use embryonic stem cells to then do the targeted gene disruption and then engineer those mice and create the first knockout mouse. So as somewhat of a recap, would it be possible if you could explain a little bit how genetically engineered mice can really help us understand the molecular pathology of a disease?

### **Dr. Oliver Smithies**

Well, maybe it's best to take a specific example. And I will take an example from my wife's work, which was done quite independently of me although she used the technique that I had a part in making. She was interested in what the genetic factors are that previous folks, some people who had developed atherosclerosis, hard – you know, plaques in the arteries and have heart attacks whereas other people don't or some other people don't. And so she wanted to try to replicate the disease in a mouse.

And now everybody at the time said that that's very unlikely to be possible because humans who develop atherosclerosis don't develop it usually until they are maybe beginning of 50 or perhaps 60 years of age before the disease develops into its mature form. And the mice only live two years, or three years perhaps at the outside.

And so it's very unlikely that they will get the disease, that's what people thought. And they also knew and the scientists knew quite a lot about the differences between mice and humans and most people have heard of good cholesterol and bad cholesterol. And if you have good cholesterol, it's fine; if you have the bad cholesterol then it's not so good. And mice naturally have very high – have very high levels of the good cholesterol and rather low levels of the poor cholesterol. So that was another reason why many scientists thought that it wouldn't be possible to generate a mouse replicating the human disease.

When my wife, Nobuyo Maeda, who was a professor here also, decided that she was going to try anyway. And she had a few ideas and in trying various ones came across the one gene that she thought was particularly promising. It's a gene that controls one of the proteins in blood. The name of the protein is apolipoprotein E and we always call it apoE so it's quite easy to remember apoE. And as she knew about this protein and then decided to see what would happen if she inactivated the gene for apoE. In other words knock it out and so she generated mice that didn't have that gene and so didn't make the protein.

And they developed atherosclerosis within six months and that on a diet that was – had almost no cholesterol and low in fat. And that mouse has been extremely valuable to researchers all over the world who want to study atherosclerosis or want to develop drugs that might help individuals who have the disease because [audio gap] and so that it has proved to be an extremely valuable mouse for the scientific community. And I believe that in fact they – the lab which distributes it now, we don't distribute the mouse anymore, the Jackson Laboratory in Bar Harbor, Maine, they distribute it and I believe it's the mouse that they sell more than any other mouse that they distribute.

So that shows you what happens and the value of that mouse is that investigators trying new things have a relatively inexpensive animal model in which they can test the new things that are maybe eventually proving to help humans.

**Marc Pelletier**

Did the – by disrupting the gene for apoE, did that reveal an important – that apoE as a gene is an important in atherosclerosis, in the process or did this mimic a secondary effect? Are there people with apoE mutations?

**Dr. Oliver Smithies**

[11:13] Well, there – that's a very good question because the answer, I was going to give refer to that, the answer to the second question, which is are there any individuals who are lacking that protein genetically and do they get atherosclerosis? And the answer is there are very, very few individuals who lack that protein. So it is, absence of that protein is not a cause of atherosclerosis in human.

But there is something more subtle than that because humans – the normal human population has three possible forms of that gene. And I don't even remember all of them, whether it's – I think apoE2, apoE3 and apoE4. I am – I don't work exactly with those so I don't remember the numbers correctly, but anyway one of those is associated with, strangely enough, and for reasons that are still not understood, with an increased risk in getting Alzheimer's, you see.

In fact, it's one of the few genes that have been securely linked to the incidence of the disease, although as I say, the connection is still very uncertain, why, it does isn't known. But it is then – it's very useful, and this is what Nobuyo has also done. It's very useful to make, to generate a mouse that expresses either of the three common forms of this gene. So she has gone further than knocking out the gene, she has changed the mouse gene or altered the mouse so that it uses the human gene instead of the mouse gene and that's the type of thing that can be done.

**Marc Pelletier**

So, a human mouse, a human mouse.

**Dr. Oliver Smithies**

Well, humanized in that respect.

**Dr. Andre Nantel**

So that...

**Marc Pelletier**

Go ahead.

**Dr. Andre Nantel**

I am sorry. So that segues actually perfectly into something that I always found fascinating in the field of using mouse transgenics and mouse knockouts is that very often you get these totally unexpected results that the best example, I teach a course at the faculty of medicine at McGill and we spend the day going over interesting mouse phenotypes and one of my favorite examples has always been, for example phos B, which is a transcription factor. So that's a protein that regulates gene expression and phos B knockouts in mice don't have any very overt phenotype except that once these mice start having pups the mother will essentially give birth different places in a cage and will never gather up the pups and feed them. The nurturing instinct in the mother is completely gone if you knockout this one single transcription factor and which leads to my question is have you ever been completely surprised by a mouse knockout or a mouse transgenic having a phenotype that you never expected and that led you do more interesting things?

**Dr. Oliver Smithies**

Oh, yes I have. I can give you a very specific example. It has a relatively complicated background so it will take me a moment or two to describe it. Many people have high blood pressure and one of the medications that they use, is very commonly and very successfully of the treatment of high blood pressure is called an ACE inhibitor and there are many varieties of ACE inhibitor that you can find manufactured by the pharmaceutical companies. So ACE inhibitors are very good at lowering blood pressure in normal or in individuals who have an increased blood pressure. And it, and so we made a mouse so that it was lacking the ACE, which is the, what that drug inhibits.

Now what is ACE? ACE is an enzyme that generates blood pressure lowering substance in the blood, I beg your pardon, a blood pressure increasing substance in the blood. So, when you have ACE, you can generate a blood pressure raising peptide substance in the blood, which is naturally at a certain level. And when we knocked out the gene coding for ACE, we expected the animals to have a higher blood pressure and indeed they did have a higher blood pressure. And so that was not surprising and – no I've got that backward, they had a lower blood pressure because they weren't, they couldn't make the blood pressure raising peptide. So the animals lacking the gene coding for ACE had higher blood pressure.

**Marc Pelletier**

Just like if they were taking a pill to inhibit ACE.

**Dr. Oliver Smithies**

Yes, exactly as you see, it was a knockout. So then we learned about the fact that out in the general population, there is a very common genetic difference that controls the level of that enzyme, of the ACE enzyme in the blood. So some individuals have a higher level of ACE than others, and that difference is very common. By that I mean that maybe the frequency of having the high form might be, shall we say, 60% of the population and 25% of the population would have an intermediate level and 15% of the population would have the lower level. So very common when you have that sort of proportions. So, very common...

**Dr. Andre Nantel**

Do you do this – do you do this often, like reintroducing mutations that you see in a normal population into your mouse models?

**Dr. Oliver Smithies**

[17:46] Oh, yes, we do indeed and that was the purpose of this experiment was to replicate the difference that we see in humans in a mouse and so we then made a mice which had the high level of the enzyme, an intermediate level and a lower line – level of the enzyme. And what we expected was that those with the high level would generate more of the blood pressure increasing per guidance, so have high blood pressure, and those with the lesser amount would have low blood pressure just as if they were treated with the drug. But what we found out is there was no difference at all in the blood pressure of these mice. So here, here we have a...

**Dr. Andre Nantel**

Wow, failed.

**Dr. Oliver Smithies**

A situation where the drug causes lower blood pressure because it inhibits the enzyme, but if you have less of the enzyme genetically, your blood pressure doesn't change. And that was a big problem and we solved it by making a computer simulation of the system. And when we made the computer simulation of the system, which I did with the help of a professor here who is a great friend of mine, Marshall Edgell. And he and I got started, and then later on I went on further with it, and we made a computer simulation of the whole system and when we did that, we found out that there was a big difference between having a small reduction in the amount of enzyme and in having the larger reduction in the enzyme which is caused by the drug. And so we recognized that the genetic difference didn't alter blood pressure because it never reduced the level of the

enzyme low enough to get into that phase of the system whereas the drug lowers the level of the enzyme much more and gets you into a place where blood pressure does change.

**Dr. Andre Nantel**

I didn't know you were in systems biology as well.

**Dr. Oliver Smithies**

Well it goes up much further than that actually because then we were contacted by investigators in France particularly by a doctor called Francois Allenjelas [ph] and Francois contacted us and said you know my studies have told me that people who have the higher level of that enzyme ACE are more likely when they are diabetic to have problems in the kidney. And therefore I'd like to take your mice that have the three levels of that enzyme, one above normal if you like and one below normal and one normal and I'll make them diabetic with a drug and see what happens to their kidneys. And he did that experiment and sure enough the ones that had the higher level of the enzyme developed the kidney problem. And that in turn led us onto a whole new set of investigations about how was that possible.

**Marc Pelletier**

I am confused. I am not confused. It makes sense, but that's amazing.

**Dr. Oliver Smithies**

Even more interesting because it turns out that the level of ACE affects how rapidly the animals get old. And so if they have higher levels of ACE they age much more rapidly than the normal animal and if they have lower levels of ACE presumably they live longer. But we have never tried that part because we have to wait rather a long time anyway with a normal mouse to give up the ghost. They live two to three years, normal mouse.

But anyway that shows you what happens when you do experiments with one purpose and end up finding that they have another valuable use.

**Dr. Andre Nantel**

That's something we are discovering a lot with mouse knockouts is that it's not like a laptop computer where you can go in and you remove one component, one resistor and the computer is dead or the TV or whatnot. Essentially mammalian systems are very robust in which you can remove one gene and there is always going to be backup redundant systems that can kick in and compensate for a missing gene. That's actually something that's very, very interesting in the field of systems biology is studying robustness using computer models to try to understand what's going on. And there is even the school of thought that this robustness is at the basis of evolution because it's the only way you can accumulate enough mutations without dying that ultimately in the long term these mutations will allow you to become better adapt at certain environmental conditions. And there is...

**Dr. Oliver Smithies**

There is one a famous observation that was made oh many, many, many years ago probably maybe 60 years, maybe even 70 years ago. And that is that if you get two copies of a gene instead of the ordinary one copy per – I am looking – in fact we have two copies. You get one from your mother and one from your father of most genes. But if you have an extra copy which happens and it can be seen in animals or in humans for that matter, then you have a spare part as it were and evolution goes much faster once you have the spare part. This is what you were talking about, Andre, I think.

**Marc Pelletier**

[23:31] But let me ask the question. We have realized with some knockouts, right, where just disrupting one gene has had a dramatic effect. Like the example Andre gave where the single gene was really – it turned out to be the mother gene. The gene that – the nurturing gene. And the ability to control with apoE, right, the – the arteriosclerosis, a single mutation not or disruption

of gene can have a dramatic effect. So I guess when you're studying a disease system this is where the knockouts or the transgenics or the humanized transgenics really are exciting because it allows us to figure that out, right. Is it possible to cure this disease because all you need to do is disrupt with pharmacological approach with a drug that one protein, right?

**Dr. Oliver Smithies**

Well that's why the pharmaceutical – the pharmaceutical companies like to use the mouse now in trying to forecast as it were or to predict which gene might help the condition they are interested in and therefore develop a drug that will do that – will affect that – the level of that product. So, gene targeting is now a constant tool of the pharmaceutical industry.

**Marc Pelletier**

You know I am very enthusiastic about the technology in that, the company here that I founded a few years ago is based on the information from a genetic knockout. So – the – this it to me I think this is somewhat of a comparative analysis in terms of the world of science. It's sort of like discovering the telescope by Galileo and taking a first look at the moon. Here now we can now in the mammalian system understand the molecular pathology of a disease. We can get lucky and identify a single gene that has an effect that can then be targeted pharmacologically or the reverse. It will lead to a long, new open field of research trying to figure out how those multi genetic traits work and that genetic robustness.

**Dr. Oliver Smithies**

But in – I am sorry to interrupt. I was going to say that combining the effects of two genes is not very easy and that's one of the...

**Marc Pelletier**

Can you do it in one shot? Are you developing vectors?

**Dr. Oliver Smithies**

I am interested, as you already have gathered, I am interested in the sorts of things that happen in ordinary people if you like. Now gene knockout is a very rare event in the human population or pretty rare. Cystic fibrosis for example is the most common gene knockout in the Caucasian or the white population. And yet it's still pretty rare though but atherosclerosis, the high blood pressure, very common. So they are due – but they're not due to any gene knockout. They're probably due to a combination of a lot of small differences and I like to put it this way that – if you look at a human, a group of humans in any room. You will see all sorts of sizes of things, people have a big nose as I do and – or a little one, they have – they're tall and skinny, or wide and short or whatever it might be. And there's nothing missing. There's nothing knocked out but they have what's quantitative differences. So I have got more and more interested in quantitative differences and it's combining quantitative differences that I have been trying to do, which is more difficult in the knockouts and gives less dramatic effect, but I think it's very...

**Dr. Andre Nantel**

This might be – this might be a right moment to actually explain to our listeners, I mean we've been using the terms mouse knockouts and transgenic mice fairly interchangeably but they're not the same thing, right. One is a lot easier to do than the other. Would you mind explaining the difference between these two technologies in simple terms?

**Dr. Oliver Smithies**

Yes, I am very happy to do the best I can. A gene – a transgenic model is that what you call the – I just lost the track for a moment.

**Dr. Andre Nantel**

Yeah, the transgenic mice and the knockout mice.

**Dr. Oliver Smithies**

Right, a transgenic mouse which has been made – which have been available now for many years, longer than the knockout mice, are made by taking some DNA which corresponds to something of interest, maybe a protein of interest, and introducing it into the mouse by injecting that DNA into another stage of development into the mouse fertilized eggs. And that DNA then in some – sometimes gets incorporated into the whole genetic material of the mouse into the genome. But it can go anywhere and so it has a great deal of randomness associated with it. They usually ...

**Dr. Andre Nantel**

It is essentially adding a gene that is over-expressed in the mouse, right?

**Dr. Oliver Smithies**

Yeah, and I was just going to say it doesn't usually go in as a single copy. It usually goes in as a string of maybe 10 or 20 copies of that gene and therefore when the mouse is born, it's over-producing that particular substance and often quite highly over-producing it. Now the difference between that and a gene knockout or a gene replacement technique by homologous recombination gene targeting is that there you can control what you put in, and so if you want to introduce a new gene or a copy of an interesting gene, you can make, you can introduce one copy of that, if that's what you wish to do and you can introduce it into the place that you want.. So it's a very much more controlled procedure. And when...

**Dr. Andre Nantel**

And now you have the third kind which is the Cre-lox system which allows you to do knockout in specific tissues by associating them with a tissue-specific promoter or a promoter that only gets turned on at a certain time during embryonic development. So there is like a tremendous level of control there in what you can do.

**Dr. Oliver Smithies**

Yes but anyway that's the difference between transgenic and gene targeting. They both have a great deal of usefulness, and they – but they do things differently. And when I make a transgenic animal I usually make a – just introduce a single copy of the – out of the extra gene into the place that were in a place, in the mouse genetic material that I know. So I know where it is and I know how many copies there are, which makes it have be more controllable.

**Marc Pelletier**

[31:16] What organisms other than, like mice have – the gene knockout is – in mice is extremely powerful and mice are great because they are small, they're easy to keep. They reproduce fairly fast and they live fairly long. Are there other organisms that have been useful in the medical field in terms of knockouts; have we started working on Macaque monkeys or chimpanzee knockouts, or are they too difficult to do?

**Dr. Oliver Smithies**

We don't know, to my knowledge nobody has done that. But – I don't know but I know all of the literature on it. There are indications now that it's going to be possible to do the – something very like gene knockout in the rat, it wasn't even possible to do a rat, which is very close to a mouse. So the mouse was particularly fortunate – the scientists were particularly fortunate in finding that it could be done in a mouse. This is difficult as I say, I know, maybe only just becoming possible in the rat. And it's not possible in many other species.

**Dr. Andre Nantel**

I think you can do a Zebrafish, lots of plants.

**Dr. Oliver Smithies**

Yes but those are different ways of doing it, aren't they? They aren't the same way.

**Marc Pelletier**

Right, but I'm really interested in the mammalian genetics, right. Because we are mammals and the extension is – we interact with mammals in an extremely important way, that we – we farm animals, there's cows for example. I'm wondering how far will this go? I mean, is there any reason why it won't work well in most mammalian systems?

**Dr. Oliver Smithies**

Well it just turns out to be difficult, and the reasons aren't clear. I mean there are lots of people would like to do it, and reproduce the system in other mammals and it just turns out to be difficult – I think the answer is we don't really know why.

**Marc Pelletier**

It would be great in primates and maybe the simpler primate systems it would be fantastic. But perhaps it's because there is – there hasn't been an Oliver Smithies to work on all the other animals.

**Dr. Oliver Smithies**

No, I don't think it's anything to do with that.

**Dr. Andre Nantel**

There's probably a cost consideration as well. It's a lot more expensive to do dozens and dozens of primates than it is dozens and dozens of mice.

**Marc Pelletier**

Perhaps, perhaps, but also it takes long hours in the lab to set up the standard protocols, and I'm sure it didn't happen the first time and it was a lot of work to get that first mouse. And when those mouse – when the genetic analysis was done on the mouse after the knockouts and the babies were born, I'm sure that there was a huge eureka in the lab.

This might be a good time to take a quick break; I'd just like to thank Audible.com for sponsoring Futures in Biotech. After this short break we can talk a little bit about the application of transgenics to other potential animals and that would be of important use to humanity. We can also talk about genetic engineering in human cells for R&D but also for clinical work and we will – there is a lot of stuff to talk about. But before we go on I'd like to thank Audible. They have over 60,000 books, radio shows, speeches, even audible versions of newspapers and magazines. So the pick of the week, let's go ahead with the pick of the week. Andre had a great idea, he suggested World War Z: An Oral History of the Zombie War by Max Brooks. It's also narrated by Max Brooks himself, Alan Alda, Rob Reiner, and John Turturro, four of my favorite people. So you want to tell us a little bit about the book, Andre?

**Dr. Andre Nantel**

Well essentially the book is – I'm a huge sci-fi fan and that's one of my favorite sci-fi book of all times. Essentially what it is, and it's told in a very original way as a retrospective history from the point of view of a journalist who's lived through let's call it simply the Zombie War. And essentially he is going – traveling over the world, interviewing the people who have had a major role to play in these world changing events. So it's very different from the usual Zombie story of four peoples trapped in a Wal-Mart and fighting away the hordes who wants to eat their brains. It's much more original than that, and in fact the whole story has been the subject of a bidding war between several production houses, I think in the end the production house that's being led by Brad Pitt, is the one who finally is going to be doing a movie out of that and it's being written up or has been written up by Joe Straczynski who is known as the writer of the Babylon 5 Series. So hopefully it'll make a great movie one day. The book itself is fascinating and I heard from Andy Ihnatko a while back that it is an absolutely tremendous Audible book.

Unfortunately myself – the way my life is organized, I don't have time for Audible books. I have enough with two hours MacBreak Weeklies and TWiT. Two of them will cover the time I get to spend at the gym every week. And because I travel with my wife I don't really read Audible book

during my commute. So, but once Marc was asking me what would make a great Audible book pick of the week, *World War Z*. I mean from everything I've read of people who've been, who have listened to it on Audible books, they have absolutely loved it and just the book itself is fantastic. So it's a perfect pick for us.

**Marc Pelletier**

Well, if Alan Alda and Rob Reiner get involved, I can tell you that's going to be one hell of a movie.

**Dr. Andre Nantel**

Oh they have awesome voices.

**Marc Pelletier**

And John Turturro, he is in *Miller's Crossing* and *Barton* – was it *Barton Fink*?

**Dr. Andre Nantel**

Yeah, probably. And it's a good warning for us scientists if we start messing up with human cells and screw up somewhere and we end up doing something like that.

**Marc Pelletier**

We'll get to that in a moment. We are actually talking about that today. So if you'd like to download *World War Z* by Max Brooks for free, simply sign up for a 14 day free trial of the AudibleListener Gold account.

It's really a win-win situation; you get to download – if you sign up, you get to download the free title. But if you decide you don't want to continue with the subscription, you can simply cancel and you get to keep the free book. So for your free book, head over to [Audible.com/biotech](http://Audible.com/biotech). We thank Audible for their support of Futures in Biotech.

[37:58] So let's start talking a little bit about those – the implications of transgenic and knockout animals, right, the genetic engineering. Here is – this is an example that my Ph.D. supervisor suggested. And he came up with this idea a few years back. And it was just a crazy idea. But it seemed to make so much sense, right.

He said why don't you knock out the prion protein from a cow? This would eliminate mad cow disease. And it's just my recollection, I think if you knock out the gene, the prion protein, which misfolds and creates the encephalitis, it doesn't hurt the animal to knock out the gene, right?

So, Oliver, what would your thoughts be on some of the applications, if we were to think 10 years from now when those – or even 20 years from now when the ability to knock out a gene into the various mammalian systems, do you think this will hit, this will be a huge contribution besides the clinical – helping us with the medical findings?

**Dr. Andre Nantel**

Or alternatively, are there any labs out there that you are aware of that are trying to apply transgenic or knockout technology to farm animals or other types of mammals?

**Dr. Oliver Smithies**

Oh, I think there are many labs that are doing both types of experiments or trying to do both types of experiments. The transgenic approach is working really, remarkably well in a lot of animal species. And so therefore, it's used already to produce substances that are complicated substances, proteins particularly, that are valuable to humans.

Perhaps one of the best examples are the antibodies that are being used for mice that by introducing into a mouse, the genes that control the production of antibodies, it's possible to

engineer if you like a mouse that instead of making mouse antibodies, makes human antibodies. They aren't anti-mouse but they now are human antibodies living quite happily inside a mouse.

And then when the mouse is immunized, it makes an antibody against whatever is being used for the immunization and that is of a human type. Therefore, that antibody is much more suitable for use in human because the human has much less chance of rejecting the antibody by making an antibody against the antibody, if you understand.

If I've made an antibody in a dog for example or perhaps that's not often used, but if I made an antibody in a rabbit and I inject the rabbit antibody into a human to try to help the human, the human will make an antibody against the rabbit antibody. And so they get away but it doesn't make – a human doesn't as easily make an antibody against the human antibody. And therefore, those antibodies are very useful. And they are used all over the world now.

**Dr. Andre Nantel**

My wife is working on a monoclonal antibody based therapy. And the amount of time and effort that they have to put in into essentially humanizing that monoclonal was tremendous. I mean now they have it in human form that can be used for therapy. But it took them easily a year and half, two years to get that mouse antibody, take all of the mouse parts of the gene while still keeping the variable regions intact, replacing the mouse regions with human regions, reintroducing them into human cells and expressing the antibodies at high enough levels so that they can purify it, it took them a year and a half, two years of constant effort. And they were helped by some very good structural biologists who could essentially model which regions needed to be changed and what couldn't be changed. Having access to a mouse that already makes human cell, human immunoglobulin is tremendously helpful.

**Dr. Oliver Smithies**

And that is available, Andre, is it not?

**Marc Pelletier**

I am wondering that is – it's really, really important because these human antibodies are now making a major contribution towards pharmaceutical. They're used as pharmaceuticals. Sometimes when a drug can't be found, if the protein doesn't want to be inhibited, just go with another human antibody that we can take, we can inject ourselves with and it will go block the function of that protein. So it's a pretty fantastic thing to be able to have a mouse that can generate these.

**Dr. Oliver Smithies**

Well, you know, you were asking about the future in a way. And I don't think that the most interesting part about gene targeting is, as far as human welfare is concerned, it's anything to do with knockout. Now I want to – I will explain that a little bit more.

I mean obviously for the reasons we have been talking about already, understanding what happens in a mouse when a gene is knockout is extremely valuable and it helps give you advise – drugs that will help a human. But nobody is going to want to knockout a gene in a human even if it were possible because they are not terribly useful.

But what we would like to do is to correct a gene that's faulty. That was the motivation behind my starting to think about doing experiments of this sort. I knew of the common ailments, genetic ailments of humans, sickle cell anemia in black people and cystic fibrosis in white people, and maybe you want to go, say, to the Mediterranean you would have thalassemia, which is a defect in hemoglobin producing gene.

All of those differences are reasonably well understood. And I had hope that it would be possible to correct those genes by gene targeting in a human but not in order to make the human produce an offspring in which the gene was corrected but in order to help the person who was involved by

perhaps altering the gene of the bone marrow so that whereas the bone marrow was producing red cells that have a type of hemoglobin that makes sickling, if you could change – correct that gene in the bone marrow of that individual, then you would have corrected their disease.

And there would be no ethical problems involved because you are treating a person who knows what you're doing and wants to have it done. And it's not messing around with anything as it were. It's just correcting an ailment. And that's what I hope will be possible.

**Dr. Andre Nantel**

[45:42] Bone marrow is a fantastic target for doing that because of accessibility and essentially the ability to eliminate the original bone marrow on the patients while you're doing the knockouts but if I remember well, the first attempts at doing, this some significant percentage of patient ended up developing leukemias because the targeting of the – I'm trying to remember if it was a transgenic?

**Dr. Oliver Smithies**

No, that was not the type of modification that I was talking about. It was a viral, introducing a virus over the correcting protein, it was not gene targeted. That's still not possible with any appreciable frequency in bone marrow cells. It's not completely impossible but the frequency is still so very low that it's not used. But I don't think that that will be true forever. There'll be – there is a new generation of scientists who are trying to improve that types of experiment. And I think it will eventually succeed.

**Marc Pelletier**

So you're suggesting that the first angle of mammalian genetic engineering applied to the clinic will be to replace a defective gene with a functional gene and as opposed to screening fertilized eggs for the defect and preventing that disease from happening in later generations.

**Dr. Oliver Smithies**

No, I think you've got two things mixed up there, Marc, if I might say that, because...

**Marc Pelletier**

That's okay, Oliver.

**Dr. Andre Nantel**

I told him.

**Dr. Oliver Smithies**

Correcting a gene in an individual in bone marrow as we were talking about has nothing to do with what babies they will have.

**Marc Pelletier**

No, I understand that, yeah.

**Dr. Oliver Smithies**

Yeah, well, you sort of made the two come into one sentence almost. So that's why I wanted to make the distinction.

**Marc Pelletier**

No, but I am thinking...

**Dr. Oliver Smithies**

Selecting embryos is a very valid way of helping people to have normal babies who have a problem gene. That's a valid way. That's not gene targeting at all. It's selecting embryos.

**Marc Pelletier**

One might have said a few years ago that we are crossing ethical lines to select embryos. And now that it seems it's such a useful procedure that it's widely adopted. But what about the next step when it becomes simple or what do you think? Do you see it ever being a situation where human embryonic stem cells are used or have gene knockouts or modifications for clinical application?

**Dr. Oliver Smithies**

For clinical applications, I don't think so, no. Not in that way, I think there are – there I can see a pathway that would use embryonic stem cells but they wouldn't probably be embryonic stem cells. They would be the sort of cell that Yamanaka just got the Lasker Award with John Gurdon –

**Marc Pelletier**

The mature cells?

**Dr. Oliver Smithies**

For demonstrating – he showed that you could take an ordinary cell, a skin cell or cell of that type and by the correct manipulations, you could get it to become something very like an embryonic stem cell, okay. And then once it became that then you could go forward with it and get to other things from it and so it's very promising way although extremely expensive at this point and not practical in any clinical sense yet.

But it points a way to starting with a cell taken from an individual, their skin cell or whatever it might be and going back and generating what amounts to an embryonic stem cell from the person's own cell, making – it goes on and on – making then some tissue from that stem cell and repairing the problem in the donor individual's own body. It's not out of sight. It's –

**Marc Pelletier**

It's –

**Dr. Oliver Smithies**

Difficult to say how long it would take.

**Marc Pelletier**

It's amazing I think...

**Dr. Andre Nantel**

So.

**Marc Pelletier**

Go ahead, Andre.

**Dr. Andre Nantel**

Technically, what is missing in our technological arsenal to be able to modify either human core blood cells or stem cells that we isolate from the blood to be able to genetically modify them and reintroduce them into a patient – I mean we have been doing this in mice for several years now. Are mice the exception? Do they have certain characteristics that make them more amenable to that type of modifications that we just can't do it in human cells?

**Dr. Oliver Smithies**

I don't know the answer to whether not do it in human cells but it's difficult. It isn't easy to do. And it's not been possible to get the frequency useful yet in situations that would be valuable clinically. And if I knew why, I would be – might be able to fix it but I don't know why.

**Dr. Andre Nantel**

Get a second Nobel Prize.

**Dr. Oliver Smithies**

Well, I would think so.

**Marc Pelletier**

[51:42] Well, I'd really like to thank you, Dr. Smithies, for joining us today. I really, really appreciate you taking the time, the second time to help us cover some of these – the future of genetic engineering. It's enormous pleasure to have you on.

**Dr. Oliver Smithies**

My pleasure to have been with you.

**Dr. Andre Nantel**

It was a pleasure to meet you.

**Marc Pelletier**

It's an honor. It's an absolute honor and I am awestruck as well.

**Dr. Oliver Smithies**

I don't know – not words you should use with respect to me anyway.

**Marc Pelletier**

I'd like to thank you for coming on today. And let me – I'll just do the close here. Dr. Smithies is a professor of pathology in Laboratory Medicine at the University of North Carolina at Chapel Hill. And he is the 2007 Nobel Laureate in Physiology or Medicine.

And as I mentioned before, his discovery on how to target a gene to an embryonic stem cell is very much like discovering the telescope by Galileo. It is – the world of medicine has been turned over on its head and opened the door to enormous amount of discoveries. So thank you for doing that by the way. I owe you a debt of gratitude as well for the research that you have done.

I'd also like to thank Andre Nantel who is a Senior Research Officer at the National Research Council of Canada and Adjunct Professor of the Department of Anatomy and Cell Biology at McGill University in Montreal.

By the way, he has a fantastic photoblog. And I urge everybody to go visit. It's [digitalapoptosis.com](http://digitalapoptosis.com). I use it as a start page. So every day I see his pictures multiple times a day and they're a lot of fun. He travels a lot for his science. And he has a keen eye for things in nature and weird things too.

**Dr. Andre Nantel**

It is a challenge to be able to post one new picture every day for five years but I do go back through the old archives and then go like, oh, my god. I mean that's the fun thing about posting every day is that you kind of see yourself growing as a photographer every year. And it does give me a break from the science.

**Marc Pelletier**

Yeah, I have seen sometimes when you've dropped your camera. He does all kinds of stuff. It's been a lot of fun. So well thank you for helping me out with the interview. Your enthusiasm is always there.

And for people who don't know, I worked with him at the National Research Council of Canada during my Ph.D. And he was very patient in answering all my questions on the bench. And by the way, say hi to Malcolm and the gang at the NRC for me.

**Dr. Andre Nantel**

Will do.

**Marc Pelletier**

I'd also like to thank the folks who made this possible, Leo Laporte, Dane Golden, Colleen Kelly who is running the Boards, Erik Lanigan and Jenny Holt [ph] at the University of North Carolina.

If you'd like a transcript to the show, they're available at [futuresinbiotech.com](http://futuresinbiotech.com). Thanks to the kind folks at Pods in Print. If you need a transcript done, even the most technical stuff, they can handle it. And you can find them at [podsinprint.com](http://podsinprint.com).

Lastly I'd like to thank Phil Pelletier and Will Hall for the opening and closing themes. For Futures in Biotech, I am Marc Pelletier. Thanks for listening.