Canadian Hemophilia Society
Help Stop the Bleeding

PODCAST SERIES

HEMOPHILIA GENE THERAPY – FROM DREAM TO REALITY

EPISODE 12

OVERVIEW OF GENE THERAPY APPROACHES

PARTICIPANT

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Intro 00:00
This is HEMOPHILIA GENE THERAPY - FROM DREAM TO REALITY, a show coproduced by the Canadian and Irish hemophilia societies. Here's your host, Brian O'Mahony.

Brian O'Mahony 00:16
Welcome to the video and podcast series entitled HEMOPHILIA GENE THERAPY - FROM DREAM TO REALITY, a coproduction of the Canadian Hemophilia Society and the Irish Haemophilia Society. My name is Brian O'Mahony, Chief Executive of the Irish Hemophilia Society. It's my great honour to introduce our guest for today's first podcast in the series, Professor David Lillicrap, who is a Professor in the Department of Pathology and Molecular Medicine at Queen's University in Kingston, Ontario, and chair in Canadian Molecular Hemostasis Research. Dr. David Lillicrap is world renowned for his research in hemophilia gene therapy, and has been a great ally to the global bleeding disorders community in helping understand the science of gene therapy. Today, he will describe what gene therapy is in 2026 and hopefully how it works. Welcome David.

Dr. David Lillicrap 01:10
Brian, thanks very much indeed. A pleasure to be with you.

Brian O'Mahony 01:13
So the first question is actually a very easy one. We're in the first generation of hemophilia gene therapies, which involve gene addition. A first challenge for you, can you describe in 100 words or less how gene therapy for hemophilia works?

Dr. David Lillicrap 01:27
Gene therapy is where we're treating hemophilia—not with a product that we inject multiple times, like the hemophilia proteins or some other non-factor replacement—it's where we give treatment once by giving a gene. There are various flavours of gene therapy, and we'll get into that later in this podcast. But essentially, this is a genetic therapy where we give a gene and make the body of the patient with hemophilia make the protein that's missing, factor VIII or factor IX.

Brian O'Mahony 02:01
So the current hemophilia gene therapies use AAV vectors. What is an AAV vector?

Dr. David Lillicrap 02:06
An AAV vector is the delivery system that allows us to deliver the gene that we're interested in being produced in the hemophilia patient, either factor VIII or factor IX. I think the mention of the word gene vectors, especially when you talk about viral gene vectors, is concerning, but what you need to remember is that we're using very small pieces of viruses. Viruses have evolved over millions of years to deliver nucleic acid DNA or RNA into cells, and so gene therapists have stolen pieces of viruses to deliver the therapeutic genes that we want the patients to receive. So a vector is basically a delivery system.

Brian O'Mahony 02:53
Would you say that a viral vector is probably the most effective delivery system for anything into the human body?

Dr. David Lillicrap 03:01
I think at the moment, that's probably true. And again, to emphasize that viruses are so good at doing this because they've been doing it for a very, very long period of time. We're now trying to deliver with non-viral vector systems. There are a number of these that are in development at the moment. Some people may have heard of lipid nanoparticles. So lipid nanoparticles have elements which are not dissimilar from viruses, but at the current time, they're still not as effective, not as efficient, as viruses. So I would say that currently, viral vectors—we'll go on to discuss particular types—are still the most effective way, the most efficient and safest way of delivering genes.

Brian O'Mahony 03:47
And just to clarify, can these AAV viral vectors transmit any disease?

Dr. David Lillicrap 03:52
They can't because they've been so manipulated, so changed that we're using about 10% of the viral genome when we use viral vectors. So, theoretically, is it possible? Really, probably not. No one's ever described a viral vector becoming infectious. I think that there's no way that you'll develop a viral infection if you're receiving a viral vector.

Brian O'Mahony 04:21
So these viral vectors are a delivery system for a factor VIII of factor IX gene. Now, in terms of the gene that's inserted into these viral vectors, where does that gene come from?

Dr. David Lillicrap 04:32
The gene is made synthetically. Ultimately, there is what we call a reference sequence, which has been derived from many healthy human individuals, and that sequence is then put together in a way that can produce the factor VIII or factor IX protein. Just a quick word about some details. The genes themselves on our chromosomes are a little complicated because they have coding sequences and intervening sequences called introns. When we deliver the factor VIII and factor IX genes, we only use the coding sequences, so we get rid of 95% of the genes, which are these intron sequences which we don't need in gene therapy. So these are normal reference sequences which we deliver to the hemophilia recipients.

Brian O'Mahony 05:25
And these viral vectors are designed to specifically target the liver. Is that correct?

Dr. David Lillicrap 05:30
Yes. You can get viral vectors which can target different cell types. So I think we're going to talk a little bit about adeno associated viral vectors, so adeno associated viruses, or AAV. There are different flavours of these viruses,

and the flavours relate to what we call the capsid, which is the particle which delivers the genome inside of it. So inside the capsid is the genome, which in our case, is a transgene for factor VIII or factor IX, and depending upon this coating, this capsid sequence, you can deliver to the liver, which is what we want to do, or you can deliver to the heart or to the brain. There are different flavours depending upon this capsid element.

Brian O'Mahony 06:17
Now, in terms of the current hemophilia gene therapies, they are not suitable to give to children. And if you look at other areas of gene therapy, it's Zolgensma to treat spinal muscular atrophy in babies, Luxturna to treat an inherited condition causing blindness. Both of these use AAV vectors, similar to the one used in hemophilia, but both are also given to young children, even babies. So why can't we give the hemophilia gene therapy to young children or to babies?

Dr. David Lillicrap 06:45
At the moment, one of the groups of patients who cannot be treated with current gene therapy for hemophilia are children below the age of 18, in fact, so adolescents and children. The reason for that is that when the vector gets into cells, it delivers the gene, the gene that we want to produce, factor VIII and factor IX, in a way that will not remain in the cell if the cell divides. So if you think about a young child, let's say a kid under the age of one or two years old, over a period of time, their liver will get significantly larger during the first 10 years of life. You don't achieve an adult size liver until you're about 12 or 13 years of age. And the way that the liver enlarges is in part by increasing the numbers of cells, and as it does that, it will dilute the effect of the viral vector. The reason that the Luxturna works in blindness and Zolgensma in the brain is that those cells, brain cells and retinal cells, are basically non-dividing cells, so once you've delivered the vector stays within those cells and isn't diluted in the same way that you get in an enlarging liver as children become adolescents.

Brian O'Mahony 08:11
How is hemophilia gene therapy administered?

Dr. David Lillicrap 08:13
Very simply, actually. It's delivered just like a blood transfusion. So we deliver this, we call it in vivo, and it's infused into a vein in hemophilia gene therapy usually over one or two hours. So it's actually a little bit anti-climactic, I think. Patients, when they receive gene therapy, are expecting some sort of major event, and it's really just like receiving an infusion of saline or a blood transfusion, and usually there are no complications at all. I think the transfusion or the infusion complications are less than 5% of patients.

Brian O'Mahony 08:54
Hemophilia gene therapies have now received marketing authorization in many jurisdictions for both hemophilia A and hemophilia B. So how safe have they proven to be? How effective have they been, and are there differences between A and B?

Dr. David Lillicrap 09:09
So let me answer the last question first, and that is that A and B are different for sure. Let's deal with hemophilia B first, because it's in some ways a little bit easier to discuss. The hemophilia B gene therapy—which was the first to show clinical promise from studies done now over 15 years ago—there are patients now who have been treated with hemophilia B gene therapy who have persistent therapeutic levels of factor IX out to 13 years. So this has been very, very stable and encouraging. That was using a non-superactive factor IX gene. We now use a gene called factor IX Padua, which will boost the expression of factor IX about eightfold. So the levels of factor nine are therapeutically very good, effective. They prevent bleeding, and they are persistent out to at least a decade and beyond. In terms of complications of factor IX gene therapy, there have been no inhibitors, and really it's proven to be both effective and safe. I think it's really very encouraging. Let's turn for a moment to factor VIII. Factor VIII gene therapy has been less effective. The reason we believe this is the case is that factor IX is made in liver cells, so when we give factor IX gene therapy into the liver, the liver does well in producing that protein. The liver doesn't make factor VIII. Factor VIII is made in cells called endothelial cells, the lining cells of

blood vessels, and so factor VIII gene therapy has been somewhat effective, but we see in particular, after about two years of expression, the levels of factor VIII fall significantly, down to about 10-15%. There have not been complications, no factor VIII inhibitors. So it's been relatively safe. In fact, it has been safe, but less effective than factor IX gene therapy.

Brian O'Mahony 11:19
Would you say that the size of the gene, the difference in the size of the gene between A and B is also a factor in in the efficacy of the gene therapy?

Dr. David Lillicrap 11:28
For sure, the factor VIII gene is significantly larger than factor IX, and so you have to engineer the factor VIII gene in a particular way to get it into the vector particle that you're delivering. So there's certainly an element about size. And as I say, that cell type that normally makes the protein is really a critical issue. Delivering to endothelial cells is not so easy, and in fact, it hasn't been done yet. And so we believe that the fact that factor IX is made in the cells we're targeting—liver cells, hepatocytes—is a major reason that factor IX gene therapy has been so effective.

Brian O'Mahony 12:08
What short term complications can arise from gene therapy, and how are they treated?

Dr. David Lillicrap 12:14
So if you think about the first year after the gene therapy is delivered, there are these fairly rare infusion reactions, say probably less than 5% of people who develop some sort of reaction as the vector is being infused. And then after that the major problem is that a percentage of patients develop short term liver toxicity. We measure this by measuring an enzyme that comes out of the liver cells called alanine transaminase or ALT and that, when it's circulating at higher levels, indicates that some liver cells are either leaking protein or are dying. The percentage of patients varies from different studies, but it's somewhere in the region of 25-30% for most factor IX gene therapy, and it's significantly higher in factor VIII gene therapy, closer to 75% of patients. Now this is a transient problem. It can be treated with steroids, with prednisone, and usually after several weeks of prednisone, the toxicity goes away. There hasn't been long-term toxicity, but it is a clinical nuisance, and some patients have taken prednisone for several months, which produces steroid side effects. So it's something that needs to be discussed with patients, and has been an issue for sure,

Brian O'Mahony 13:43
What type of long-term side effects are possible, and has there been any evidence of these occurring to date?

Dr. David Lillicrap 13:50
The major concern about gene therapy in the long term is whether the vector sequences end up producing problems in the host genome. So let me just say a few words about AAV. AAV, when it gets into the nucleus, the majority of the genome of the vector persists outside of our chromosomes. So within the nucleus, we have the human chromosomes, and they are not routinely invaded by the vector sequences. The vector sequences remain as what we call episomes, or extra chromosomal structures within the nucleus. And actually, that's why, when the cells divide, the nucleus also divides. And that's why you dilute these extra chromosomal elements. However, because we give such large vector doses, although less than 1% of the vector sequences get into the chromosomes, less than 1% of about a trillion particles is still a large number, and so there are within the host chromosomes, vector sequences, the material that we're delivering the factor VIII and IX transgenes get integrated into our chromosomes, and that happens in a random way, not targeted anywhere, and so far, there has been no evidence that that causes complications like long term risk of cancer, for example. So in patients that have been studied to date, there's been no evidence that these integrations are producing long term problems like malignancies.

Brian O'Mahony 15:34
Can you talk briefly, David, about the monitoring required in the first year and then long term after the first year?

Dr. David Lillicrap 15:41
The monitoring in the first year is relatively intensive. Patients need to have blood drawn weekly for the first three or four months to look out for these liver enzyme elevations, the ALT elevations. So for the first three or four months, there's fairly intensive blood drawing, and then out to one year, you still need to have blood drawn to make sure that the liver remains healthy. Beyond that, there needs to be annual ultrasounds carried out, actually, perhaps every six months, an ultrasound needs to be done to make sure that the liver is healthy and that there's no evidence of any tumors being formed in the liver. In addition, you need to get blood drawn to measure the factor VIII or IX levels, and probably every year, to make sure that there's been no evidence of inhibitors forming, although there's been no history of inhibitors being formed. Actually, one case in one factor VIII study that I'm aware of. But inhibitor formation has been a rare complication in gene therapy so far.

Brian O'Mahony 16:45
Now, a second generation of gene therapy for hemophilia B is now starting clinical trials. It's described as a novel gene editing technique. David, can you explain how this differs from the first-generation gene addition process?

Dr. David Lillicrap 17:00
So gene addition is where we're giving the factor VIII or IX genes, the normal genes, into patients with hemophilia. Gene editing is where sometimes we are giving the normal sequence, but we're putting it into a precise place in the human genome. So the difference with genome editing is that we are purposely inserting genes or correcting the mutant gene through an editing process. It's a more precise way of delivering hemophilia gene therapy than the current way with AAV vectors, where we're substituting the normal sequence which remains in the nucleus. But remember, generally outside of chromosomes, gene editing is where we're making changes in the host chromosomes.

Brian O'Mahony 17:55
And what advantages might this technique have?

Dr. David Lillicrap 17:58
The advantages are that we're specifically and precisely making changes in the chromosomes based upon systems like the so called CRISPR system. Most people have heard about CRISPR now, and CRISPR is a way where we make changes in the host chromosomes in a very precise, localized way. So it means that we can deliver, for example, into a region of the genome, the host genome. Let's take an example. There are some genome editing studies ongoing where we deliver the factor VIII or factor IX gene into the albumin gene. Why would we do that? Albumin is a protein that is made at high levels in the liver. So it's a very good target to make a large amount of protein by targeting this gene. And so you can do this with genome editing. It doesn't actually alter albumin levels. There's no concern that you're going to have a problem because of reduced albumin, but it means you're making the factor VIII or factor IX protein in a region of the genome which will be at high levels from liver cells.

Brian O'Mahony 19:14
Does this also come with increased risks?

Dr. David Lillicrap 19:17
The major concern is that the editing, although it is precise, there may be some low level of off-target manipulation of the genome. In other words, although you're precisely editing the albumin gene, there will be a small risk, actually a very small risk, that there could be editing taking place at other chromosomal sites. That can be minimized by optimizing the CRISPR system, and also, if you edit cells outside of the body, you can actually make sure the off-target events are very, very small and therefore re-infuse cells in which the off-target manipulation events are screened out.

Brian O'Mahony 20:06
And is it not the case, David, that you could also use this gene editing technique in children?

Dr. David Lillicrap 20:11
You could, indeed, because if you're making changes within the chromosomes, as the liver got bigger, those chromosomes would be maintained, and it would be beneficial in a way that current gene therapy with AAV vectors is not.

Brian O'Mahony 20:25
So theoretically, this should last for lifetime. Is that correct?

Dr. David Lillicrap 20:30
Correct. Exactly. You could actually do this in a six-month-old. I mean, we're obviously a long way away from that happening. But theoretically, once you make a precise change with genome editing that should be persistent throughout the lifetime of that individual. The other thing to say about genome editing is that, depending upon the delivery system that you're using, you might be able to … or you should be able to re-administer the genome editing mechanism, which you cannot do currently with AAV gene therapy. With AAV gene therapy, the immune response to the vector is so profound, so strong, that you cannot go back a second time or a third time with gene therapy using the current AAV system. With genome editing, especially if you're using lipid nanoparticles, there is the possibility of re-administering if you get an initial sub-optimal response.

Brian O'Mahony 21:34
There's an entirely different approach, a B cell approach, being taken by another company, which is in early stage clinical trials. Can you describe how that works?

Dr. David Lillicrap 21:43
This is a very interesting mechanism. The company that's doing this is working on what they call B cell medicines. So B cells are a form of lymphocytes which eventually, when they get mature, turn into plasma cells, which make antibodies, high levels of antibodies in their system. This company is removing B cells from patients with hemophilia B. It just happens to be a lot of B's here. So, hemophilia B is factor IX deficiency, of course. So they remove the B cells from the hemophiliac. They isolate these B cells in vitro, so in a petri dish, outside of the body, they then edit those B cells by inserting the normal factor IX gene from humans, although it's actually the Padua gene, so it's the hyperactive factor IX sequence, and using the CRISPR system, they place that into a safe harbour in the chromosomes, so there's a gene that they can put this into which they know will not cause problems. Once they've edited the B cells, they grow up the B cells in a milieu, in a group of growth factors, which turn the B cells into plasma cells. These plasma cells then are making lots of protein, which now includes factor IX Padua, and then they re-infuse these cells back into the patient. But this is all done outside of the body. It takes several weeks to do this, and eventually, the edited plasma cells are sent back to the patients and can be infused by a peripheral vein infusion. And the cells find their way back into the bone marrow, actually, so they home to the place where they normally are, which is in the bone marrow of the individual. The other good thing about this is that if the initial infusion gives a low level of factor IX, so it's sort of partially successful, you can re-infuse those cells. So they keep some of the cells in storage, so they can go back a second or maybe even a third time to increase the levels of factor IX.

Brian O'Mahony 24:03
So it's both titratable and re-dosable?

Dr. David Lillicrap 24:07
Exactly. If this works … if it works … it would be really very, very interesting. And of course, the other question would be, well, would this work for factor VIII? We don't know, but certainly they will be looking at this if their factor IX studies are positive,

Brian O'Mahony 24:24
If we can go back for a moment to hemophilia A. David. Hemophilia A gene therapy has been approved by regulatory bodies such as FDA, EMA and Health Canada, among others, but concerns over durability have discouraged wide adoption. It's marketed now in only three countries, USA, Italy and Germany, and its future is uncertain. So in your view, what breakthroughs are needed to make hemophilia gene therapies viable in the future, and how soon might we see some of these new approaches?

Dr. David Lillicrap 24:54
Factor VIII is always difficult. I think that everyone who works in the gene therapy—or actually in molecular biology—knows that factor VIII is hard, and in part, it's because of this strange thing that it's made in a small subset of the endothelial cells, these blood vessel lining cells. The current form of gene therapy has worked. There are patients out at seven years post AAV gene therapy with factor VIII who have therapeutically useful levels of factor VIII between 10 and 20% so to say that it failed is actually incorrect, but it has been disappointing for sure. I don't know that anyone's going to try additional AAV therapy with factor VIII. I think that there's been a general sense that it's been disappointing, and so any further commercial uptake of this, I'm not sure that's going to happen. It may be that factor VIII gene therapy will jump straight to some form of genome editing. And indeed, there are at least a couple of examples of companies who are using editing approaches now for treating hemophilia A where they edit particular regions of chromosomes to insert the cDNA sequence, the coding sequence of factor VIII. So I think it's unlikely that they will get into licensed products much sooner than another three or four years at the earliest.

Brian O'Mahony 26:23
David, you've worked in this field for many years. How do you see the future unfolding?

Dr. David Lillicrap 26:28
I think that factor IX gene therapy is already working. So to me, the uptake of that should actually be high. It's getting there now with the one gene therapy product that's licensed in several jurisdictions. But I think if you ask me what's going to happen in 10 or 15 years, I think increasingly, there will be uptake of some form of genetic therapy, be that substitution gene therapy, or in the longer term, some form of genome editing. I would be surprised and really disappointed if in 10 to 15 years, a significant proportion of hemophilia patients would not be considering some form of genetic or genome editing treatment.

Brian O'Mahony 27:14
What's your definition of a significant proportion?

Dr. David Lillicrap 27:17
Let's say a third of patients.

Brian O'Mahony 27:20
David, thank you very much. We really appreciate you always being ready to help educate and inform the hemophilia and bleeding disorders community. It's been a great pleasure to talk to you today. For more information on gene therapy, we invite you to check out our other podcasts in the series, Hemophilia Gene Therapy - From Dream to Reality. Thank you very much.

Dr. David Lillicrap 27:40
Thanks, Brian.

Signoff 27:42
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