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Evolving Gene Therapy: A Deep Dive into 3rd-Generation Lentiviral Vectors

Announcer:

You're listening to *Clinician's Roundtable* on ReachMD. This non-certified educational series is produced and controlled by ReachMD, and is intended for healthcare professionals only. This episode is sponsored by Bluebird Bio, and now, here's your host, Dr. Matt Birnholz.

Dr. Birnholz:
Welcome to the *Clinician's Roundtable* on ReachMD. I'm Dr. Matt Birnholz, and joining me to explore 3rd generation lentiviral vectors in gene therapy is Dr. Donald Kohn, who's a distinguished Professor at the University of California Los Angeles. Dr. Kohn, it's great to have you here with us today.

Dr. Kohn:
Thank you. It's great to be here. Excited to talk about this.

Dr. Birnholz:
Yeah, absolutely. Me too. So just to start off, I'm interested in doing some level-setting here on lentiviral vectors, or LVVs for short, and I understand that there is quite an interesting developmental history for this technology. Do you think you could just give us a quick primer on LVVs and how they've evolved within the field of gene therapy?

Dr. Kohn:
Sure. So lentiviruses are a class of viruses that are in the bigger family of retroviruses. That whole family integrates its genome into the chromosome of the cell they infect. And so they're very good as gene delivery vehicles because you can just put your gene into those and let them bring the gene into the cell and put it in the chromosome. And for things like stem cells or T-cells that are going to divide a lot, you want to have the gene of interest in the chromosome of the cell. So every time it divides, it gets passed onto the daughter cells. And so the initial work in the 80s began with using a mouse-based retrovirus as sort of the cousin of lentiviral vectors. And then in the mid-90s or so, lentiviral vectors were developed.

Dr. Birnholz:
As I understand them, lentiviruses have a number of interesting characteristics that set them apart for which they became really strong vector candidates. They have a capacity to permanently integrate their transgenes into host cell genomes as well as being able to infect both dividing cells that you mentioned as well as slow dividing cells, is that correct? Are there other characteristics that set them apart?

Dr. Kohn:
Yeah, so the two things that you mentioned are important. The non-dividing or the slowly dividing cells means they can go into things like liver cells or brain cells that, again, the earlier mouse retroviruses couldn't do; the mouse retroviruses need their target cell to actually be dividing and going through mitosis to be able to get to the DNA and integrate. Lentiviruses can go through the nuclear membrane because it has pores in it. They can get through there to the DNA to integrate even in a non-dividing cell. One of the points to make in the very beginning is the basis for the lentiviral vectors that are commonly used is the HIV/AIDS virus. So it's a virus that infects people and integrates its genes into T-cells, and it expresses some of its genes that cause the T-cells to dysfunction. And so the vectors were made by taking out all the genes of the virus and just using the ends of it, sort of as a carrier for genes of interest.

Dr. Birnholz:
Why don't we talk about aspects of the generations of lentiviral vectors. I understand we have our 1st generation, 2nd generation, and

3rd generation. Can you help tweed those apart for us and the evolution of these generations of lentiviral vectors?

Dr. Kohn:
The very first lentiviral vectors basically took out the envelope gene of the virus and put a gene in it to be carried. So it was mostly very much like HIV, except it had one gene missing. But the concern was that all it needed was a crossover with a wild type of virus to make an infectious virus. And so the successive generations were made to make it all look less like a lentivirus and more like expression plasmas in a sense. And so the vector just has sort of the regulatory ends of the virus and none of the parts of the virus that coded for the protein. And so with the successive generations, the vectors got cleaner and cleaner in terms of having remaining parts of HIV.
And then it was sort of a co-evolution of the vector and the packaging systems. These are like binary systems where instead of a virus that carries all of its information to make new viruses, you take that information out of the virus and you use it to carry the gene of interest, but to package it, you have to provide the genes that make the viral shell proteins at the same time. And so typically, plasmids are encoding the vector with your gene of interest and encoding the proteins that make up the virus component, and those plasmids are all transfected into a packaging cell line that will copy those plasmas into RNA—RNAs are made into protein and the virus particles assembled. And the successive generations make less and less—basically zero—overlap between the different plasmid components. So the possibility of a recombination to make live virus has been zero to the present time as far as I know.

Dr. Birnholz:
So while all generations to this point, you know, coming in at the third generation, are still rooted in the framework of HIV, it sounds like the significant modifications have helped enhance their functionality, and have helped enhance their utility in various gene therapy applications. Is that right?

Dr. Kohn:
Yes. That's right. So an example would be in the virus, it has all the genes that make the proteins all in a row. In the current 3rd generation packaging systems, the proteins make the virus shallow by themselves, and then a regulatory protein is in another separate plasmid, and there's no sequence overlap. So the possibility of a recombination is really very low. Earlier generations that might have all been in one plasmid. And so fewer hypothetical recombination events would have been needed to make an infectious virus.

Dr. Birnholz:
We turn that in the form of replication competence, isn't that right? Among these viruses that, in this context of lacking accessory genes, coming from the wild type lentiviruses, there's new safety measures now that could get introduced and you can minimize the possibility of replication competence?

Dr. Kohn:
Yes. And so all that goes into the patient cells, if you're treating T-cells or stem cells or whatever or you're giving them in vivo the virus, all that gets into them is the genetic information for your gene of interest. All the genes to make more HIV proteins are back in the packaging cell line. And so there's no way the virus can make more of itself and start a spreading infection, which is replication competent. So these vectors are replication incompetent and are completely unable to replicate themselves.

Dr. Birnholz:
Are there other distinct advantages over alternative vector systems when we're talking about the 3rd generation LVVs?

Dr. Kohn:
Right. So compared to the retroviral vectors, for example, lentiviruses have a bigger carrying capacity. So you can get up to about 6 kilobases of gene to be carried. So that's getting to be a pretty big gene or promoter whereas the retros are more about 3 or 4. So there's that. There's the property we talked about of going into non-dividing cells, and if you just compare them head-to-head, for example, the retroviruses to the lentiviruses to put genes into T-cells or stem cells, the lentiviruses do it better. If you're targeting hematopoietic stem cells, when we take those out of the body and culture them to put in a gene, every day that we have them in culture, we're losing stem cells. You know, they differentiate. And with a retrovirus, you have to have the cells in culture for about 4 days to get the cells dividing to take up the vector. Lentiviruses can kind of do it overnight. So you could have the cells and culture for a day and a half, 2 days. And that 2 days versus 4 days really makes a difference in the stem cell survival, the number of stem cells you're giving back to the patient. So that, I think, maybe the most important.

Dr. Birnholz:
Excellent. Thank you. That's a fantastic description. And for those just tuning in, you're listening to the *Clinician's Roundtable* on ReachMD. I'm Dr. Matt Birnholz, and I'm speaking with Dr. Donald Kohn about 3rd generation lentiviral vectors in gene therapy. So, Dr. Kohn, we talked about risk and we think about the safety profile aspects. What are some common adverse events associated with 3rd generation LVVs that we need to keep in the top of our heads?

Dr. Kohn:
Yes. So the risk of insertional oncogenesis still exists. So the retroviral vectors that were used earlier that actually caused leukemia in a few patients, they did that because they carried very strong enhancers at their ends, and that allowed them to make a lot of virus when they're infecting, but that allows them to turn on a nearby gene. And so what made the lentiviral vector safer was that realization, and they're made without the enhancers in the ends. So the ends of the virus are called long terminal repeats, and you could make them so they don't have strong enhancers and drive your gene off just a weaker promoter; you still get enough product, but lower that risk of turning on nearby genes.

Dr. Birnholz:
And what kind of monitoring is typically employed? I imagine it might be a lifelong process to monitor for either hematologic malignancies or other insertional types of changes over time in patients post-treatment. What is the standard protocol for that in your experience?

Dr. Kohn:
So in the research setting, the FDA rule is that patients need to be followed for 15 years if they get a vector that's going to persist, whether it's an integrating lentivirus or even an AAV vector. And so typically the clinical trial might monitor the patient for two years, then they're asked to participate in a long-term follow-up study where once a year they basically need to see a physician, have an exam, have a blood test, you know, a blood cell count. And that's all that's typically required.

Dr. Birnholz:
For a subject of this depth that always feels like we are barely scratching the surface, I'm delighted by the time you've been able to spend to this point to help us uncover a little bit about 3rd generation LVVs. Before we go though, any other takeaways, thoughts, or parting concepts for our audience that you might want to impart?

Dr. Kohn:
Well, I guess I'd just say, you know, for a gene therapy, there are now a lot of different tools. There's lentiviral vectors that we've talked about. There's AAV, adeno-associated virus, that has been curative in patients with spinal muscular atrophy and hemophilia. There are all these new editing techniques with CRISPR-Cas9 and base editing and prime editing. And it's so great to have these different tools and different diseases and different organs need different approaches. And so we're probably in the second inning of gene therapy or something. We now have multiple effective therapies. But I think we'll get more and more. And one of the big goals for my area of blood cell diseases in hematopoietic stem cells is to be able to do it in vivo. And rather than taking the stem cells out, we'd be treating them, giving chemotherapy, and giving them back, delivering the gene. So I think there's still a lot to come in the future that will make it better at treating many, many genetic diseases.

Dr. Birnholz:
Those are definitely exciting and forward-looking takeaways that I think our audience is really going to resonate and be excited about. I very much want to thank my guest, Dr. Donald Kohn, for joining me to help explore 3rd generation lentiviral vectors in gene therapy and look at the broader space in a novel and unique way. Dr. Kohn, it was fantastic having you on the program. Thanks so much for your insights.

Dr. Kohn:
Great. Thanks a lot for having me. My favorite topic.

Announcer:

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