

Critical Assays for Delivering Potent Gene Therapies

Lauren Drouin discusses the tools and techniques behind successful viral therapeutic delivery.

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Researchers built the foundation of gene therapy delivery on viral biology.^{1,2} Today, scientists engineer enhanced delivery technologies, such as novel adeno-associated virus (AAV) capsids, and optimize therapeutic potency with precise droplet digital PCR (ddPCR) technologies, such as Droplet Digital™ PCR (ddPCR™) Assays from Bio-Rad for assessing viral titer and beyond.¹⁻³

Lauren Drouin is a gene therapy scientist with unique expertise in the field's progress from preclinical development to the clinic. As the director of analytical development within the Genomic Medicine Unit at Alexion AstraZeneca Rare Disease, Dr. Drouin develops accurate, precise, and robust analytical methods for gene therapy delivery technologies, including novel AAV capsids. In an interview with *The Scientist*, Dr. Drouin shared the principles, benefits, and challenges of developing viral vectors, and discussed how improved tools and techniques such as ddPCR technologies strengthen the present and future of gene therapy research.

Q: How did you become involved in gene therapy research?

A: When I was young, one of my very close friends was diagnosed with a rare genetic disorder and we did not understand what that meant for her at the time. I became interested in the idea of a treatment for this type of disease, and I stumbled upon the concept of gene therapy. I ended up going to the University of Florida for my undergraduate degree where I studied microbiology. I had some really great professors there, specifically Dr. Jim Maruniak who was my virology professor, and I learned about adeno-associated viruses for gene therapy. In graduate school, I joined a couple of different laboratories that were studying AAV biology. I studied under Dr. Mavis Agbandje-McKenna, who was a preeminent structural biologist for AAV studies, as well as Dr. Nicholas Muzyczka. Collectively, these experiences played a role in propelling my career towards the study of AAV for gene therapy purposes.

Q: How does AAV-mediated gene therapy delivery work?

A: Viruses evolved to deliver their own genetic material into their host. We are using that same principle, but instead of using viral



Lauren Drouin is a gene therapy scientist with unique expertise in the field's progress from preclinical development to the clinic. Photo credit: Nina Bauer, PhD, MBA

DNA, we substitute a therapeutic transgene. The virus has to bind to the cell, become internalized, and traffic to the nucleus. Once it gets to the nucleus, the capsid enters intact and then breaks open to release the packaged genome, which contains the therapeutic transgene. From there, that therapeutic gene will

form a circular piece of DNA or in some cases, integrate into the patient's genome. Then every time the cell replicates, it will make a copy of that DNA and express the therapeutic transgene. The big advantage of using a viral-based delivery approach is the ability to target specific cells. That is one thing that the lipid nanoparticle field is moving toward, but they are not quite there yet.

Q: What challenges do you face when developing AAV capsids?

A: With some of the novel AAV capsids, we do not know very much about their long-term stability. One of the other main challenges is that it can be more complicated to develop a potency assay for new capsids because we do not know what cell lines they can transduce naturally. Potency is by far the most complex attribute to assess for gene therapy products because we need to measure several major steps involved in the therapeutic mechanism of action. We have in vitro assays that target specific steps in this pathway, all the way from infectivity and mRNA transcription to protein expression and functional protein activity. These assays will be developed and assessed as part of the product development cycle, and this often begins with a gene expression assay (measuring mRNA transcription). For that, we can extract RNA from cells that have been transduced with the viral vector, perform reverse transcription to generate cDNA, and quantify the resulting cDNA using ddPCR technology.

Q: How do researchers in the AAV gene therapy field benefit from ddPCR Assays?

A: I think for the AAV gene therapy field, it has become the gold standard for vector genome titering. This assay is how we determine the clinical dose given to patients, so it is critical to get it right. If we administer too much, that could cause severe adverse reactions in the patient. However, at this time, gene therapies are not redosable, which means that if we do not administer enough vector to elicit a therapeutic benefit, patients cannot be retreated. It is essential that the vector genome titer is determined accurately. Previous to ddPCR technology, the field was using quantitative PCR (qPCR). In theory, these methods work very similarly but I think qPCR has some drawbacks. It relies on a standard curve,

so it does not measure an absolute titer. qPCR is also more sensitive to inhibitors in the sample buffer, so if there are certain additives, it can change the titer of the product. Besides that, there is a higher amount of variability in the qPCR assay compared to ddPCR technology. It is a natural progression that we have come to a better assay now, with more absolute quantification and less variability. Over the last few years, I think the field has moved wholeheartedly toward utilizing ddPCR technology for determining vector genome titer.

Q: What excites you the most about the future of AAV gene therapies?

A: We have been applying learnings from previous classes of drug modalities to AAV manufacturing and analytical testing to improve product yield and quality, while reducing cost of goods. We are at the point where the ball is rolling in gene therapy and we are making a positive impact on patients' lives. We have seen several new approvals in the last year and the U.S. Food and Drug Administration is predicting ten to fifteen new cell and gene therapies per year by 2025. I am really excited for where the field is going. I think we are learning more about this tool and how we can use it to treat rare diseases, but also hopefully move that into some larger diseases in the future, such as Parkinson's disease, Alzheimer's disease, and different cardiovascular diseases.

References

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This interview has been edited and condensed for clarity.