CELL & GENE THERAPY INSIGHTS

THE LATEST DEVELOPMENTS
IN VIRAL & NON-VIRAL VECTOR
MANUFACTURING

SPOTLIGHT

INTERVIEW

Applying learnings from the bioprocess development of Luxturna™

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BIO

Q As you reflect on the historic approval of Luxturna™ last year what are the key lessons you take forward on the vector manufacturing side of things?

FW: It was a real honor and privilege to be involved in the development and approval of Luxturna!

It was just great to participate in the founding of Spark Therapeutics, help build and work together with the team that brought it over the finish line in terms of the clinical and product development, including the complex validation and submission processes. I'd also like to emphasize and acknowledge the key role of Children's Hospital of Philadelphia (CHOP) and the team there led by Katherine High, and the University of Pennsylvania team led by Jean Bennett and Al Maguire, where the discovery research, IND-supporting studies, clinical program and manufacturing originated and was completed through the pivotal trial.

Upon reflection, I would say a few things on the emerging successes in gene therapy. Firstly, diligence and persistence are important! I think we're seeing now on an ongoing basis the proof of principle of human gene therapy, building on years of lessons learned – it's a matter of getting the gene that is known to be missing to the right location to achieve the right level of expression of the therapeutic transgene, and importantly long-term durability. These are all important objectives, and not easy.

"...we need to be proactive in regard to manufacturing process development and vector product characterization."

With Luxturna, both the dose and patient numbers are relatively small, so we didn't face a significant manufacturing capacity issue. The quality attributes and QC around the manufacturing was very important and perhaps more challenging – especially considering we are at an early, pioneering and rapidly evolving era in human gene therapy development and commercialization.

So I would say that we need to be proactive in regard to manufacturing process development and vector product characterization. There are great advances we still need to make to mature this exciting technology. I'd also emphasize the importance of having good multi-lot experience as early as possible to effectively support later stages of product development and validation.

Finally, I'd say that for many clinical indications in gene therapy in general, product development can happen quickly, accelerating beyond what are considered normal timelines for CMC development for traditional biologics. For example, if the mechanism of therapeutic action for an investigational gene therapy product is solid, the clinical benefits can be transformative, so the urgency to move forward quickly is there. And that can be a big challenge on the manufacturing side: there's potentially less time available for development – less of an interval between the early clinical phases and what might be a pivotal study, for example. It is very important as we move forward to anticipate the need for more mature stage CMC development earlier, including process and assay validation activities.

Q You oversaw bioprocess development for the first AAV gene therapy product to receive market authorization in the US – could you share some of your key learnings on the regulatory side?

FW: The first thing to bear in mind is gene therapy is still very much an emerging area. While the manufacturing processes being used are built on the basics of processes and procedures established for other

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biologics, like vaccines and recombinant proteins, there are certainly distinct differences. So we're still learning things and innovating bioprocessing technologies for gene therapies. There still remains a great gap to address in terms of ability to manufacturing sufficient amounts of recombinant viral vectors for the ever-expanding group of diseases that gene therapy can potentially address in a transformative manner. To drive home this manufacturing capacity technology gap, consider that monoclonal antibodies are made in lots with yields measured in 'kilograms' of purified product; in contrast to recombinant AAV vectors that can currently only be made in 'gram' quantities – one gram of rAAV corresponds to approximately 10^17 vector genome containing particles, we need to aim for three logs higher as a technology objective. This manufacturing capacity gap must be addressed to realize the full potential promise of human gene therapy. There exist similar gaps to address for viral vector characterization methods and analytics; I'd emphasize in this category especially dose-determining titer methodology, and potency assay development.

We're still in an era where we need to be very scientific in our approach: in terms of analytics, we have to be scientifically innovative about how we can move forward. For example, some of the assays being used for gene therapy product characterization, such as measurement of vector packaged residual DNA impurities from the production cell or other DNA components, are unique product-related impurities for viral vector-based gene therapies. Potency assays are very complex for gene therapeutics as well. These are issues that really have to be carefully analyzed and I'd say well trained and innovative teams need to be prepared to get in there early, tackle these needs, and anticipate significant technology requirements going forward.

Regarding all aspects of CMC for gene therapy product development in general, I would reference (FDA Commissioner) Dr Scott Gottlieb's comments at the Alliance for Regenerative Medicine luncheon in May this year (2018): to paraphrase, he suggested that perhaps 80% of the challenge currently facing gene therapies is on the CMC side. I would encourage readers to access a copy of Dr Gottlieb's comments, which I believe are available on the internet. This important perspective strongly emphasises the major challenges represented by CMC in this field – adequate resources and attention need to be provided!

Q Much is made of the current viral vector capacity manufacture bottleneck. Where does the answer lie for you?

FW: It is a challenge. There are a number of excellent CMOs out there but many are just getting up to speed now for gene therapy products,

and I think that demand on the gene therapy clinical development side is very, very large. So my sense is that we are at a stage where demand is outpacing supply right now in terms of CMO provision of clinical vectors. This results in potential delays in clinical development programs, and may impede certain teams with promising potential clinical programs – I'm thinking especially about strong academic groups – due to the high costs involved.

In an analogy that has been presented by others, I would draw parallels with where monoclonals were perhaps 20 years ago, when the CMC technology was still fairly early in its development curve. We can consider monoclonal CMC technology to be 'mature' today, with kilogram quantity manufacturing capacity and 'well characterized product' status for analytics; by contrast gene therapy CMC technologies are still at an earlier stage, perhaps midway along that technology evolution curve.

So I would say that more time and innovation is what we still need. If we fast forward 10 years from now, I believe we will be past the current manufacturing capacity bottleneck. Technology will have matured and be more easily able to make larger amounts of vector in more standardized platform methodologies, and more comprehensive and standardized analytics will be in place. But right now, the CMC gaps we see are just a fact of life in the field. More investment on the CMO side is going to be needed, and we're going to have to have a little bit of patience as we work through this bottleneck.

CMOs are very important right now with all their new initiatives coming through in gene therapy – for academics and also early stage biotech companies, it's just too expensive for many, if not most, to set up in-house capabilities beyond very early stage CMC operations. So there's going to have to be ongoing technology development and optimization on the CMO side. I'm confident that will happen, driven by market forces and the enormous promise of these transformative therapeutics – but we need to be aware of these challenges to keep this field moving forward quickly.

Q Staying on the topic of CMOs, what have your years of experience taught you about key success factors in outsourcing vector bioprocessing?

FW: I think that's a very important question. I would go back to the fact that we're still early in the technology development curve – early to mid-stage – so there are a lot of things in gene therapy manufacturing, and in CMC in general, that are less robust than we'd like them to be.

CMOs are trying to accommodate a lot of different programmes, and there is less standardization out there than would be ideal, so it is vital

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that there is as much expertise as possible on the client side: it's never just a 'plug-and-play' situation, there are always challenges arising. The CMC aspects are complex: so the scientific technology challenges are still quite significant. It is really important that a specific investigative product being developed by an early stage biotechnology company, or which has come out of an academic translational research operation, has the strongest possible expertise around it. Gene therapy vector design – I'd consider as the first 'C = chemistry' in CMC - is very important. Our understanding here is still evolving and there are some key features – critical quality attributes – that need to be better understood. Critical quality attributes for purity, potency, safety and long-term transgene expression relating to factors including human immune responses to the gene therapy vector need to be defined and controlled. And I'd further emphasize overarching quality systems, compliance, consistency and standardization.

In addition to this requirement for strong client-side expertise, effective technology transfer and efficient communication are also vital to improve the probability of success and tighten timelines in moving through clinical development.

Q What are your thoughts regarding potential pathways to further improve viral vectors – by incorporating non-viral technologies, for instance?

FW: The strongest argument for the use of viral vectors is they're so efficient at delivering their payload – the nucleic acid component of the gene therapy product – to the nuclei of target cell. In my view, that's why viral vectors have dominated gene transfer programs to date. It is a very complicated pathway to follow, the journey from a site of injection, to the target cell surface, through the cytoplasm to the nucleus, and then stabilization of an expression cassette in the nucleus – be it extrachromosomal (rAAV) or integrated (rLenti). Viruses have evolved over eons to be very efficient at delivering a piece of DNA along a complex pathway to where it needs to be to enable efficient expression, and it is extremely difficult to 'engineer' non-viral systems with comparable efficiencies. But viral vectors are certainly not perfect, and I'd say one of the biggest problems we need to address relate to barriers caused by the efficient human immune response to viruses – and hence to virus derived vectors – which have similarly evolved over time to block infection of wild-type viruses.

"CMOs are very important right now with all their new initiatives coming through in gene therapy..."

Are there good non-viral replacements, such as nanoparticles? I think that's a great concept, but with a long way to go. I expect that with enough time and advanced technologies we can achieve some of the efficiencies inherent naturally in viral vectors. But it strikes me as very challenging, a little like the concept of predicting protein 3D structure from a primary sequence. For the next decade anyway, I tend to think more in terms of how we can improve the viral vectors we have now – how we can build on their inherent efficiency, and yet address some of their challenges.

"...can we modify our viral vectors using non-viral vector methods and technologies to address such issues?"

One major category of challenges when using viral vectors for efficient gene delivery relates to immune response. There are a couple of aspects to consider. One is pre-existing immunity, and this is a significant issue when talking about *in vivo* administration – for example, with certain AAV vectors, systemic administration can be prevented by even modest levels of pre-existing antibodies. For most AAV serotypes, there tends to be a range of pre-existing antibody titers population so that *in vivo* gene transfer efficiency is likely to be variable. Since a first administration of an AAV vector will cause the formation of antibodies – the normal response to exposure of the human immune system to a viral antigen – so the opportunity for a second administration is generally prevented, at least by systemic routes of administration. Another feature of viral vectors that we need to better understand, manage and control relates to immune responses that may limit long-term therapeutic gene expression after successful target cell transduction. I would say that closing remaining gaps in our understanding of the quality attributes and features of viral vector products relating to human host immune responses, and further innovation in vector design, have the potential to markedly improve gene therapy vectors in these regards.

So these may be opportunities where we can think of perhaps adopting a hybrid approach: can we modify our viral vectors using non-viral vector methods and technologies to address such issues?

Q With Axovant Sciences, you are involved in working with a lentiviral vector platform in an ***in vivo*** therapeutic application. Lenti does seem to be making a comeback as a viable ***in vivo*** option – what's different this time around?

FW: I think safety has been a recurring theme when using more complex viral vectors such as a lentiviral vector. Of course, the parent viruses are obviously more significant safety concerns when

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compared to AAV. But we have seen incredible progress in the *ex vivo* area – the licensure of CAR-T therapeutics Kymriah and Yescarta for cancer. Those therapies have now been administered to many patients now and are having a very profound effect. Having so much more of this real patient experience and solid safety data has resulted in *in vivo* gene therapy developers looking at lentiviral vectors again in a variety of therapeutic areas and indications, including CNS, ophthalmology and hemophilia.

Lentiviral vectors have about twice the packaging capacity – genetic payload space – of AAV, so there's a real advantage there: there are many expression cassettes that exceed the packaging capacity of AAV – I think those are great opportunities for rLenti vectors. Another advantage with rLenti is that such integrating vectors retain therapeutic transgene expression efficiency in dividing cells – a feature lacking with the canonical rAAV gene transfer vector.

So I think that increasing confidence in the safety of using lentiviral vectors for *in vivo* administration, combined with their larger packaging capacity, form a strong argument for further development of the lentiviral approach for *in vivo* gene delivery.

But we should emphasise that recombinant AAV and recombinant lentiviral vectors are both excellent approaches for viral vector-based gene delivery, they have unique respective advantages. I would say that different indications should leverage the relative advantages of one or the other, depending on their specific requirements – they're both great platforms for transformative new medicines!

Q Gene therapy product candidates are now showing promise in diseases with comparatively large patient populations, such as Parkinson's and AMD. How will we manage the potentially huge increase in manufacturing capacity that will be required when such therapeutics reach the market?

FW: I would go back to the fact that the technology is evolving in viral vectors right now. We're at a point where we're only part way through this technology evolution, optimization and development. Again, very analogous to the situation with monoclonal antibodies from the 1990s until now, where we started with murine antibodies that often caused efficacy-limiting immune responses, characterized with relatively primitive methods, and produced at modest milligram per liter quantities in cell culture, and innovated across the board to today, where we have fully humanized monoclonal products, considered 'well characterized' using

sophisticated analytical and quality control methodology, and produced at robust gram+ per liter amounts.

The great success of mAb's to address many unmet medical needs came about as the result of, among other factors, the innovation and diligent product design evolution as well as manufacturing process and analytical methods development focused on this class of complex biologics. To me, we are meeting the same challenges and following the same trajectory in our ongoing technology efforts with gene therapy vectors to support the enormously promising new therapeutic paradigm of gene/nucleic-based medicines.

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