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Gene Therapy Analytics: Identifying and Overcoming CMC Challenges in Gene Therapy

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ABOUT

Susan D'Costa is the chief technology officer of Alcyone Therapeutics. In this presentation, D'Costa discusses how companies can identify and overcome CMC challenges in gene therapy, including understanding and monitoring DNA constructs; identifying starting material challenges; prioritizing potency assays; and the impact of viral vector delivery and development of capsid tropism.

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Ben Locwin, Black Diamond Networks: Welcome, Susan. Before we start off can you give me a bit of background on yourself?

Susan D'Costa: Thanks, Ben. I'm a molecular virologist by training and I've trained on various viruses starting with the basic biology and translating into gene therapy. I started working translationally with gene therapy about a decade ago, where I started in process development at contract development and manufacturing organization Brammer Bio, now part of Thermos Fisher. That's where I learned about process development and quickly grew in the field and translated my expertise and knowledge into QC, where I spent time working on viral vector analytics and assay qualification. I then moved back as the head of process and analytical development. Later, I transitioned out of the process development realm into being a subject matter expert for technical program design before I moved out of CDMO completely to my current company, Alcyone Therapeutics.

BL: Let's get started. In the context of DNA constructs, what do you think are the most important considerations in terms of delivering consistent productivity?

SD: That's a really profound question, right? It's easy to say that you transfect cells with the right constructs, and you should be able to make a good viral vector. But depending on what process one uses to build out the product, the final productivity, the efficacy, and the impurity profile will change. The cliché is the process is the product. More and more we're walking away from that. As long as we have good characterization assays to be able to understand the product, the process doesn't need to be the product. Regardless, focus should be placed on the best technology one tries to put in place with a specific indication? For example, when I'm thinking about neurological diseases, I want to understand what is my efficacy? What is my productivity? What is my cost of goods? I want to be able to build out

the best technology and understand what is the best technology to build up my process. Then, I would layer on the analytical capabilities of that platform. Especially for companies that are working on multiple assets, it's important to be able to build out a platform process. For example, for the same serotype of AAV, you don't want to reinvent the process. You want to be able to improve your cost of goods. You want to make the process, characterization, and validation streamlined. A quality by design approach is essential, as early as possible during the drug discovery phase.

BL: In thinking about starting materials and how they dramatically influenced the entirety of the process, what are some of the challenges most often seen?

SD: When I think of starting materials, the two main components I think about is one, the cell component, because most of the viral vectors that we make are made in bioreactors, by using certain kinds of cells. The idea is to be able to find a clone or a population of those cells that would be ideal for the production of our vector. In the case of HEK293, not all the HEK293 cell lines that you can find are going to produce comparable to each other. So there might be a reason why your team decides to work on subcloning those—identifying a clone that has superior productivity as compared to the population, or working through and understanding what media and what supplements are critical to be able to grow these cells up in bulk, or the mass you need, before you are able to produce your vector. Then, often, when you're thinking about your cells, you start with a research cell bank during process development. You identify what your process needs to focuse on all the design of experiments for scaling up. But in parallel you want to build out a master cell bank. That master cell bank is not necessarily going to produce the same as your research cell bank, unless you're taking the time to test it in process development before you're ready to go into a clinical manufacturing phase. That is important when you're thinking about cells and showing that the bank is consistent and robust no matter which vial you pick out of that bank—and that vial has been tested in process development prior.

The second bucket I'm thinking about is putting in some kind of DNA to yield a viral vector. That DNA can be transiently transfected, the cells can be stably transfected to have the gene of interest as well as the helper functions inserted into the host chromosome this DNA can be introduced via the helper virus system where you can build your starting materials by building virus vector constructs, and this could be the baculovirus system or the HSV system. No matter what approach one takes, one needs to make sure the starting material is built in a quality-controlled manner. For example, if you take plasmid specifically, whether you're using a two-plasmid approach or a three-plasmid approach to build your AAV, you need to make sure that you're looking at what the initial constructs look like. A lot of times these were built up in academic labs, where you cut and paste, you take the original plasmid, construct the backbone, take out the old gene of interest and throw in your new gene of interest. What that can do, if you're not careful, is leave hotspots for recombination. If you find that those hotspots recombination are going to give you a higher stochastic event to get to replication competent AAV, it's too late in the conversation by the time the AAV viral vector is tested for release. Now you have to go back and rebuild those constructs to be able to take away those hotspots. So, when those plasmids are being constructed, or if you're getting those plasmids from academic labs, doing really good QC to confirm that there aren't any remnants that don't need to be there is critical. This is also in the case of adenovirus helper plasmids, because a lot of the older constructs that were used as late as a couple years ago, had hexon and spike protein left in there. Unfortunately, both of those proteins are very immunogenic and we know that AAV does encapsidate not just the therapeutic DNA, but also remnants of plasmid DNA and host cell DNA. If all of this DNA and maybe protein is being expressed in the viral vector construct, that could be really deleterious in terms of safety. So, trying to build out the right plasmid constructs is really important.

BL: It sounds like a common thread through all of this, or maybe the architecture around it, all ties back to quality by design, and how the quality and QC of each step has direct influence on the steps that directly succeed them downstream.

SD: Correct. In parallel, to think about productivity, one must also pay attention to the small-scale processes. What are the residual impurities looking like? Are we carrying forward any of these impurities? A lot of times, it's easy to wait until a final process lot step to look at things like replication competent AAV, or look at how much residual host cell DNA,

or how much residual plasmid DNA is left in the viral vector. By that time, it might be too late to change the process and be able to solve for this.

BL: What have you seen are the most critical or influential issues regarding impurities?

SD: I bucket impurities into process impurities and product impurities. With process impurity you can find operations that will help to improve that, but when I think about product impurities, that is the bigger challenge. The challenge is, how can you differentiate a vector, a capsid, that contains DNA that is of the same length, but not therapeutic DNA? How do I separate that out? Because, often, the charge may not be that different to be able to use ion exchange. The size, the weight, the density will not be different when I'm using density gradient centrifugation. Which means trying to minimize how much gets encapsidated in the upstream process is the way to go, rather than maximizing purification processes downstream. Also, how do I manage those impurities? It's very important and, again, there's a quality by design concept where you think about your quality target product profile. What is your indication? What is your viral vector going to be used for? How much viral vector do you need? And therefore, what is the risk of the impurities that you're bringing in? If you're using a high-concentration, high total load of virus that needs to go in systemically, clearly, you don't want a whole bunch of empty capsids there because that will cause a serious immunological output that you're not interested in. Safety wise, it's dangerous, but also efficacy wise. You probably are transfecting cells with these empty capsids or with non-therapeutic DNA and you don't want that either. So, the quality target product profile converts into what the quality attributes of the product needs to be.

BL: Thinking about potency, do you have any best practices with regard to potency assays?

SD: Yes. The two main aspects I think about when I'm building out a process is the dosing assay, because that dosing assay needs to be good and tight to be able to get the different doses when you're doing a dose escalation study. Very closely married to that is the potency assay, because if we cannot determine that the vector lot you're working with is efficacious, it's really difficult to be able to identify how it's going to work in the clinic. Unfortunately, potency assays are generally very complex, either because they're bioassays and inherently challenging to keep precise because of the variability, or there is no easy way to express what you need to express—whether that's to show transduction, or to show expression or any of the other matrices. Identifying as early as possible innovative ways to work on potency, whether that's an expression assay or a functional assay, and then building it out as robust as possible is critical. During process development, potency assay development, qualification, and validation needs to go on. Of course, you don't validate the assay until much later in the lifecycle of process development. But regardless, having an assay that you could quickly be able to test different constructs, be able to use during the end process, or for process development, or just in general to be able to say one lot compares to the other, that's critical. As part of that potency assay, can your assay tell you what a 20% potent lot looks like? Can your assay tell you what a 300% potent lot looks like? Building those fences to be able to understand that, I can then say my specification needs to be 50 to 200% of the reference, I can do that, because I've shown that the assay is dynamic and quantitative.

BL: What are your thoughts about viral vector delivery? Will the current development and focus on capsid tropism be enough to successfully target the right targets in the human body?

SD: When I think about all the clients that I worked with throughout my lifetime, the most common theme was identifying ways the capsid can make the tropism work, or the capsid is able to cross a barrier, like a blood-brain barrier to get across into the CNS. In addition, there's a lot of conversation around how to make the promoter express more once it's transduced. How can I get more expression of my target? That, and then how can I get the right amount of vector, the right amount of dose to the right region in the human body, and to the right cell? That, to me is a more orthogonal approach. The capsids and the engineering of the capsid, absolutely makes a difference. But when you think about the holistic way of developing CMC, every time you build a new capsid, you have to customize a bunch of new aspects that you have to put in place. As a field, we need to continue to exploit and explore orthogonal ways of doing this.

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This case study was presented at Evaluating Biopharma's virtual networking and educational event *Gene Therapy Analytics: Implementing Better CMC Strategies*, which included two additional presentations and two interactive networking sessions.

Details of future events can be found here.

You can watch Susan's presentation in full and on-demand here.

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