

EXPERT ROUNDTABLE

Evolution in AAV process development: 2022 and beyond



ALEJANDRO BECERRA

Principal Applications Scientist and Global Purification Technical Lead, Thermo Fisher Scientific

Dr Alejandro Becerra is a Principal Applications Scientist and Global Purification Technical Lead. Alejandro has over 14 years of experience in downstream processing and customer support having worked as Purification Team Manager and other bioprocess engineering roles prior to joining Thermo Fisher Scientific in 2018. Dr Becerra is a subject matter expert in preparative chromatography with expertise in the development, optimization and scale-up of antibody, recombinant protein and viral vector purification processes. Alejandro holds a PhD in Chemical Engineering from Cornell University.



MATTHIAS HEBBEN

Vice President of Technology Development, LogicBio Therapeutics

Matthias Hebben has been serving as vice president of technology development at LogicBio Therapeutics since February 2019. In his role, he is leading the CMC efforts, including vector core, capsid optimization, process development, analytical development and clinical product manufacturing. Before that, he served as director of technology development and head of bioprocess development at Genethon for 6 years. Before that, he occupied several positions at Vivalis (Valneva), Intervet Shering Plough and Virbac. Matthias has a PhD in molecular biology and a MSc in bioprocess engineering



MICHAEL MERCALDI

Senior Director of Downstream Process Development, Homology Medicines

Michael is the Senior Director of Downstream Process Development at Homology Medicines. He is responsible for leading the development of Homology's purification and drug product manufacturing processes for their gene therapy and gene editing programs. He has held positions in process development throughout his career at MedImmune/AstraZeneca, Merrimack Pharmaceuticals and Codiak Biosciences before joining Homology. He holds a BS in Chemical Engineering from the Rensselaer Polytechnic Institute and a PhD in Biochemical Engineering from Tufts University.

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In 2019, BioInsights hosted a popular [roundtable video](#) and [article](#) with leading experts in AAV vector process development. Two eventful years later, we're bringing our panel back together to discuss how viral vector manufacture is evolving and what we can expect in the future.

Q Let's begin with regulation: what are the key areas of uncertainty for the gene therapy industry at present, and how have these changed since our last discussion?

MM: Overall, we have been very pleased with the regulatory agencies and how they are approaching gene therapy.

It's a very rapidly evolving space, and they're trying to learn and work with manufacturers to make these therapies work better.

We would like to see a little bit more guidance on impurity levels – what are the impurities we should be looking for, and what are the acceptable levels that we need to achieve? For example, what is a safe level of empty capsids? That's an area where companies like us can proactively work with the agency to help figure what is a safe level of these impurities.

We would also like a bit more structure and guidance on CMC activities during development. Gene therapy moves very fast, and if you're treating a pediatric condition or ultra-rare disease, you may only try to register at Phase 1 data. The agencies are very proactive and want to work with you on getting that done, which is great, but we'd like to see more definition on what CMC activities need to be pulled in earlier. That will help us as a

CMC organization to plan better, meet those demands more easily, and ultimately get these therapies out faster.

MH: There is more and more guidance for gene therapy products, which is always very useful. For me, the big changes that occurred for the last two years were the severe adverse events that have happened in several clinical trials. This is certainly going to change the scope of what is acceptable in terms of purity and product quality, as Michael mentioned.

It is going to be very important to understand what is happening in clinical trials and what could be causing toxicity – whether it be from capsids, impurities, or other sources. It's an open question today.

AB: As a vendor, we don't get deeply involved in regulation, but we need to be aware of it. For example, if guidelines change around empty/full capsid analysis, we need to understand if the current tools we offer are sufficient.

Q Potency is one issue that has grown dramatically in significance since we last spoke. What for you are the key learnings for the field from the various tribulations suffered by industry players, and what would be your advice regarding the timing of potency assay development in particular?

MH: The big challenge is that the infectivity of AAV is totally different between *in vitro* and *in vivo* and even between different animal species. This

makes a potency assay a challenge – it’s very complex to be able to identify or characterize the mechanism of action when you have to use massive amounts of vectors to be able to transduce a cell *in vitro*. That raises questions about the sensitivity of a potency assay. Again, because you have to use such a huge amount of vector *in vitro*, it is not necessarily representative of what is going to happen in a human body. It’s very difficult to know whether a small change in the vector efficacy from batch to batch can be detected in your potency assay.

Over the last two years, we have seen several examples of companies that have faced some setbacks with regulatory agencies because of some issues with potency. From what I understand, most of these issues were associated with changes in the manufacturing process. That means there are still unidentified critical parameters in manufacturing processes. We need to perform a lot of investigations to be able to better characterize these processes.

It seems obvious that developing a potency assay should be done very early in development. However, it’s difficult to be comfortable developing such an assay before you know if your intended mechanism of action will work in patients. It’s difficult to know what parameters you will need to follow *in vitro* in your assay. Developing an assay before you know what is going to be the effective dose in patients can be also very tricky.

To sum up, while it is important to start developing the potency assay as soon as possible before the clinical trial, people need to

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- Michael Mercaldi

understand that the assay will be a living protocol and will need to be adjusted over the course of clinical development.

MM: It’s becoming clear that agencies, especially the FDA, are now asking for multiple potency assays – infectivity, gene expression, and a cell-based potency assay. They feel that the three assays tell different parts of the story. There is also an increasing expectation that these assays will be available earlier in the lifecycle.

We’re prepared for that at Homology – we have platform methods that we can quickly develop infectivity and gene expression assays for any kind of construct. Cell-based potency does require construct-specific work and, as Matthias was saying, that needs to happen very early in development. Once we identify a construct, we start developing the assay, so we have it in hand for our critical IND-enabling lots. That means we have all the data that the agencies are expecting and gives us a lot of confidence that we’re making a quality product.

Q Turning to challenges for process development and product characterization stemming from reduced development timeframes – for instance, in the expedited regulatory pathway scenario: what are the implications for process and assay development and what steps may be taken to help avoid issues at the BLA stage?

MM: If your organization is trying to pursue an accelerated pathway, there are going to be expectations from any regulatory agency that you need to pull in some later-stage activities earlier in the development than you normally would. Organizations need to be ready for this – if you think you're going to be trying to register on Phase 1 data, you need to prepare to do a lot of these BLA-enabling activities at your IND stage. This puts a lot of pressure on a CMC organization because you need to do all this work plus balance timelines. How we've addressed this at Homology is by investing heavily in our platform and building an excellent analytics team with a large suite of analytical assays and deep knowledge of our constructs.

We are then able to leverage this platform and analytical knowledge, to pull in a lot of these activities very quickly or leverage

previous construct knowledge that can give the agencies information that they need.

MH: I think this expedited regulatory pathway is a breakthrough in the field of regulation. It's a fantastic opportunity, most importantly for the patients, but also the industry.

But as Michael said, it means that you must set up your company as a commercial-phase company from the start. When you're a very small company trying to develop new technology, you don't know if you're going to be successful in the clinical trial. It requires money and time to be able to have everything ready before you start your clinical trials.

AB: From our side, we're seeing the same trend toward greater investment in process development. Organizations are spending more time upfront, building stronger analytics and process characterization.

Q What have been the key advances in AAV manufacturing technology over the past two years, and how might they continue to reshape vector bioprocess moving forward?

AB: I think in terms of technologies, progress has been a little slower, but we are seeing the utilization of different approaches with the existing tools. For example, potentially using shorter residence times and shorter bed heights for capture. And similarly, utilizing different approaches to make full capsid enrichment separation easier than with traditional gradients.

MM: I wouldn't say progress has been slow but I agree with Alejandro that the focus has been on taking existing tools developed for the recombinant protein or other therapeutic space

and making it work for AAV. For example, Thermo Fisher Scientific developed the excellent affinity resins that are now used quite widely.

Other advances in the past two years include a more aligned process across the industry, consisting of a harvest step, an affinity step, an anion exchange step, and final formulation. When more companies use that same basic process, I think we're going to get a lot more learning and understanding about what those products are and how they work in that process. Plus, they will be more scalable.

Another thing that I've noticed a lot of companies do, including Homology, is to transition from ultra-centrifugation to anion exchange chromatography to remove empty

capsids. For me, this is a key step to bringing these therapies to a broader patient population. Because now we have a scalable manufacturing process that can be executed well with our existing toolset. It means we can support larger bioreactor sizes and make more vector for broader patient indications. At Homology, we can now run a 2,000-liter bioreactor and have a very scalable high-throughput process using hematographic techniques.

Where I think the field needs to focus next is trying to develop new technologies for virus and nanoparticle separations. Vendors are already working on this, which is great. They're being very collaborative, and we work with a lot of great vendors on trying to develop these technologies.

MH: There have been some advances in transient transfection of HEK293 cells, which remains the most popular and reliable process to manufacture AAVs. Notably, new transfection reagents have improved yields significantly.

As mentioned by the other panelists, affinity chromatography is a fantastic tool to achieve high recovery and high purity of AAV capsids.

Enrichment of full capsid is still a challenge – some serotypes are very easy to purify but others are much more challenging. It's something that everyone is focusing on today, so certainly the next big step will be in that area I believe.

Q Toxicity issues have been much discussed in the AAV field of late – what do you see as the key pathways forward on the bioprocess side if the field is to address these concerns?

MH: As I mentioned earlier, there have been several recent toxicity events that have led to serious adverse events or even deaths. What shocked me most is the very early toxicity described by Jim Wilson's team recently, which seems to be correlated with complement activation, meaning that the capsids appear to immediately induce a toxic event. For me, I think that suggests the industry should consider decreasing the total amount of capsids in gene therapy products. That means, on one hand, removing empty capsids but also working with more effective serotypes or engineered AAV capsids that can be used at lower doses and are more tissue-specific.

At LogicBio we are putting a lot of effort into developing different methods to enrich for full capsids, and we have implemented a capsid engineering platform called sAAV_y, which allows us to decrease the effective dose of our vectors.

MM: I think Matthias gave a great overview of what the field is experiencing right now. For us, we have one product in the clinic, HMI-102 for phenylketonuria, and so far, we have found that to be very well tolerated.

Beyond our observed clinical data, we leverage a lot of understanding of toxicology

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- Alejandro Becerra

and immunogenicity from our colleagues

across the organization, outside of CMC. For example, we've used mouse models to study the impact of empty capsids on potency, and through *in vivo* testing, we are starting to understand the impact of our capsids on immunogenicity. That allows us to tailor our processes to ensure a safe and effective product for our patients.

AB: Matthias mentioned full capsid enrichment as an important issue to address, and that's something we continue to work on internally and in collaboration with our partners, whether it's with existing tools or trying to develop new ones.

Q Can you comment on how and why the outsourced versus in-house vector manufacturing picture has developed over the past two years, and what future trends do you foresee in this regard?

MM: At Homology, we've taken an in-house manufacturing path, and we believe this has allowed us to quickly build up our pipeline.

Besides just manufacturing, internal process development and analytics also play a huge role, in that we're able to spend a lot of time, effort, and energy on developing and understanding our platform and products. This has allowed us to make significant improvements in both overall yield and purity. With internal capability, you have better control over your development pipeline and can dedicate more resources towards the internal manufacturing and development model.

AB: During the last roundtable, one of the challenges that came up was the restriction in the amount of AAV that can be produced globally. Two years on, we are seeing more organizations taking a similar approach to what Michael described. It's certainly a trend to see more and more

in-house manufacturing, not only for the AAV but also plasmids. Plasmids are certainly one of the critical raw materials and have their own challenges in terms of supply. From our side as a supplier of these tools, we want to ensure we can provide the materials necessary at the right time.

MH: I have very mixed feelings about this question. It really depends on the indications that the company has in its portfolio. We are seeing more and more improvement in the manufacturing process. If you target a very small patient population for ultra-rare disorders, maybe you can supply the patient population with a very limited number of batches per year and it may not be the highest priority to have a manufacturing facility that is not going to be busy all year long.

But if you want to address a broader population and you need to manufacture one batch per week, clearly that's a different story.

I'm not overlooking the fact that today the CDMOs are very busy, and the queues are very long. But I think there are more and more players in that field.

Q The COVID pandemic means the world is a very different place today compared to last time we spoke – what for you are the key ongoing issues for the gene therapy field that relate (directly or indirectly) to the pandemic, and what best practices have you sought to introduce at your respective organizations to counter them?

AB: It's definitely a different environment than two years ago. The pandemic put a significant strain on the suppliers, as some of the tools that are important for the cell and gene industry are being used for vaccines and therapies against COVID. It has prompted lots of suppliers to invest in expanding those manufacturing capacities, but since it wasn't planned it has taken some time, and it varies for specific products.

For the products I work with, namely chromatography resins, we were fortunate that we haven't really seen any impact on affinity resins, but other types of chromatography have been affected.

Fortunately, we had already been investing in an expansion of our existing facility and the build-up of a new one. We have accelerated those activities but it's still going to be sometime early next year before we can get back to normal delivery times. For now, we are working closely with our customers to improve delivery times where we can.

MH: It has been very complex for everyone, with shortages of everything from pipette tips to filters. In R&D that is manageable because you can always change your material and switch from one supplier to another. In GMP, it's a different story. You cannot do change control for every single raw material you would have to modify at the last minute so it can be a critical issue.

We have been very lucky at LogicBio that we have not been impacted at all in terms

of GMP manufacturing. I know others that have been in a very bad situation as a result of shortages. I think this pandemic has shown how vulnerable the field is in terms of the supply chain. I hope there will be a solution to the shortages soon, but also that there will be a lot of work to anticipate the next big events and prevent shortages in future.

This pandemic has not just had huge impacts in the lab and the manufacturing space. For clinical trials it has been a real challenge – due to the risk of hospital systems becoming overwhelmed, clinical trials have been put on hold. At LogicBio we have been very lucky because we have been able to maintain very close contact with the clinical specialists, and able to continue to identify patients and have smooth enrollment of participants as soon as hospitals were able to be open. Thanks to that, we were able to start our first clinical trial, for pediatric patients with methylmalonic acidemia, this summer.

MM: I think we have a little bit of a different perspective because we have so much internal capability. We definitely experienced some supply chain issues, but we have a great supply chain team that anticipated this to some degree, and they were able to work with the vendors proactively to make sure we were able to have the supplies we need to continue manufacturing. Consequently, we had no interruption to manufacturing, which was great.

In addition, our process development teams built a lot of redundancy into our process, to allow us to use alternative chromatography resins filters and so on. That means if we do have any supply chain issues, we're not dependent solely on one vendor.

I think the pandemic has highlighted weak points and forced companies to plan more for worst-case scenarios. Organizations will now aim to anticipate these shortages and make sure they have enough material in stock to ensure that manufacturing is uninterrupted, so clinical trials or commercial supply are not impacted. I think our organization has been able to do that pretty successfully.

MH: I agree that companies need to anticipate more in advance in this new world. But the issue remains – how do you manage the expiry dates of some ingredients when you have to stockpile things? Unfortunately, anticipation cannot solve all these problems.

MM: Absolutely. We do development stability for two years – in fact, we have enough material to go out to 36 months or longer. It comes back to the internal manufacturing, where we can plan out what batches we want to do and when, to align with clinical trials and expiry.

Thank you – it's interesting to get two contrasting perspectives from companies who have adopted different manufacturing models. It's clear from our conversation today that AAV process development has evolved significantly since we last met in 2019, and this has been a valuable update on the emerging advances and challenges.

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