

EXPERT ROUNDTABLE

AAV vector process development: achieving high purity and high yield - experiences from the frontline



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Meisam is Head of the gene therapy downstream process development at Biogen. In this role, Meisam oversees development of purification processes to support Biogen's AAV gene therapy portfolio. Meisam has 8 years of experience in developing downstream processes for protein biologics and viral vectors at different stages of development. Meisam received a PhD in Chemical Engineering from The Pennsylvania State university.



MICHAEL MERCALDI
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Michael is the Director of Purification Process Development at Homology Medicines and is responsible for leading the development of Homology's purification manufacturing processes for their gene therapy and gene editing programs. He has worked purification 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.



MATTHIAS HEBBEN
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Matthias has served as VP Technology Development since February 2019. Before that, he served as Director of AAV Technology Development and head of bioprocess development at Genethon for 6 years, where he managed the design and scale up of manufacturing processes for AAV and LV vectors. Prior to his role at Genethon, Matthias was Director of the Virology Unit at Vivalis for 4 years. Before that, he occupied several roles in the animal health industry at Intervet Schering Plough and Virbac between 1999 and 2008. Matthias has a PhD in molecular biology from the University of Nice Sophia Antipolis (France) and a bioprocess engineer degree from the University of Strasbourg (France).



ORJANA TEROVA
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Orjana is Senior Product Manager for Purification products within the Bioproduction Division at Thermo Fisher Scientific. Orjana has a M.S. in Chemistry from University of New Hampshire (in 2008). She also has 11 years of industrial experience at Thermo Fisher Scientific. Her expertise includes a strong technical background in small molecule and bead chemistries, new product development and commercialization. In her current role Orjana is responsible for managing globally the POROS Chromatography product line, including life cycle product management, implementing programs to drive business growth strategy and support of the global sales organization.

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Q What are the key challenges today primarily related to scalability and large scale manufacturing of viral vectors, and what should we expect in the future as more therapies move to commercialisation?

MM: The challenges center around overall process productivity - both upstream and downstream. We're currently able to produce enough vector for our clinical products with high quality and high yield, but we see overall process productivity as a real opportunity for us. It's easy to imagine that we are where the antibody space was about 25-30 years

ago, with a world of sub-1 g/L titers. We're able to make high quality product, get it into the clinic, but there's a huge opportunity to really increase our productivity. This can be done through innovation and understanding of our product, our process, and biology. Hopefully in the long term we will be able

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to produce larger amounts of the viral vectors needed for these therapies and move to curing larger indication genetic diseases.

MH: I agree - there's a big leap to make in terms of productivity and in particular the upstream part of the process. Because clearly the demand for AAV vector is absolutely huge in terms of dose per patient for gene therapy; and therefore it's essential we tackle inefficiencies in our process, especially upstream where I believe we can improve things.

MB: Maybe I can focus this more on scalability of the downstream processes. As many of you will know, historically processes such as graded ultra centrifugation have been used to purify AAVs, but that's not a very scalable process. Over the past few years a lot of companies have tried to move away from these centrifugation processes and focus on chromatography-based separation for purification of AAV.

As a result, more and more chemical chromatography and filtration platforms are becoming the main tools used for the purification of AAVs, with the added benefit of being scalable processes with which we have a lot of experience of these in operations from protein biologics process development.

Having said that, there are still some gaps with regards to downstream purification of AAVs. For example if you were using a membrane filtration or chromatography, especially monolith chromatography, there are some challenges in terms

of the scalability of those devices. These devices come in very discrete sizes, so in a lot of cases scalability could be a challenge and this could present a real bottlenecks for downstream processes.

OT: I definitely see that as the field has been growing and more potential therapies move from early phase through to commercialisation, that there are some manufacturing and scalability challenges. However, there are lots of opportunities to optimise upstream processes to produce more viral vector and reduce bottlenecks downstream.

I think efficient upstream and downstream purification solutions or strategies to generate clinical product with high titer and potency and purity is very important to advance the field of gene therapy.

Keeping this in mind, from the Thermo Fisher Scientific bioproduction standpoint, we are enabling this paradigm shift in serving the gene therapy field through the Capture Select technology, the basis of which is a single antibody domain, heavy chain only, high specificity ligands for affinity chromatography. And with that the Poros Capture Select AAV Affinity Resins have already proven to be an essential purification platform for a wide range of AAV serotypes, enabling high purity, high productivity processes with fewer purification steps, while offering process consistency and scalability.

Q The issue of yield is certainly something we hear a great deal about in this sector, would the panel agree that this is a pain point for each of you, and what would be your approach to address this?

MB: As the other panellists mentioned earlier, the low cell culture titers we are seeing in AAV manufacture are a challenge and bottleneck in processing and manufacturing of these vectors.

In terms of yield, the downstream processes are often low yield. One of the areas to highlight is that upstream processes producing low titer AAVs often do so due to a need for a volume reduction in the process. We typically use some sort of

filtration process to reduce volume to maintain your processing time within a reasonable processing range.

That really contributes to loss of yield for AAV. Particularly for AAV, we all know that it's very prone to absorbing to multiple surfaces, as a result you lose a lot of product as a result of non-specific binding to product contact surfaces. Now there are ways around it - you can perhaps use detergents in your intermediate in order to mitigate this absorption to surfaces. But there's always some sort of binding issues for AAV.



And finally, since the cell culture titers are low and we're dealing with large volumes, often times we also tend to underutilise our chromatography steps, for example through under-loading our columns, and that can result in some yield loss throughout the process as well

MH: Generally speaking, when you look at the biology of AAV, the productivity is not that low compared to other viruses that are used in the vaccine industry. For AAV we are looking at a best case productivity of about 1E5 vector genomes/cell, which is not that bad compared to other viruses.

However, there's no doubt we would all like to have higher productivities in the manufacture of AAVs, and for that to be achieved we have to work at the cell level, to try to work with the cell lines, to make them more productive. I think this is the very first step, to really play with the biology of the virus and the production cells.

Regarding upstream processes, to be able to have a more cost effective scale up would be ideal, because of course the second option to improve the productivity is to work with higher volumes. And of course for the downstream processes the less vector we lose the better it's going to be. But all this considered, AAV manufacture isn't as problematic as say other viruses that are much more fragile, such as enveloped viruses.

MM: That's a good point. For us, really it comes down to two things: if we have continuing process understanding and process improvement, we're going to hit that goal. It's just a matter of time. But you have to do the work. You have to put in the time and effort to really understand what are the challenges and how you're going to increase your productivity.

Another critical element is that gene therapy developers need to collaborate with vendors, to develop more tools geared towards the space. Understandably, most of the tools are developed for the antibody market given it's such a large share of the vendor space. But that presents challenges for us such as finding things like filters, consumables, tubing, GMP processing systems that can handle the smaller volumes we get through the downstream process. If we have tools that are custom designed for the gene therapy space, then I feel we can reduce a lot of these non-specific yield losses, or any other mass balance

issues we get. So then we can end up delivering more products to patients when we're done with our process.

OT: completely agree with the panel's points and in particular as Michael mentioned, we think it's essential to collaborate with our customers and other therapy developers to make sure we are understanding the right pain points so that we can develop and commercialise the tools that the field needs to improve yield and purity.

As Matthias mentioned, there's a lot of effort upstream to improve that, but then also downstream, simpler processes where you're not losing product every step of the way, trying to capture your product in one or two steps at high yield purity becomes important. So we need to have the right solutions as vendors for that, so we can enable our customers to meet their productivity targets.

MM: I would echo that. We're a big believer in collaborating with vendors, because vendors only get a little bit of a window into what we're dealing with. The more you open up to vendors, the more you work with them, the more you collaborate, that's how you're going to drive a lot of innovation. It's really a symbiotic relationship. If you keep everything too closed off, the vendors won't really know what the pain points are and how to develop better products for us in the end.

OT: And finally I think for gene therapy we can hopefully get to scalable and platformable processes just like those that exist in the mAb world, so a lot of these challenges with yield and productivity will be addressed.

We are constantly trying to figure out the real challenges for this sector so that we develop the right tools to enable the industry to thrive and get more therapies to the patients, faster.

Q How does affinity chromatography improve or affect your AAV production?

MH: I think affinity chromatography is a fantastic tool for AAV vectors, especially the POROS resins from Thermo Fisher, because they have a very high capacity and specificity, In the past I've worked on processes that were based on only one single affinity chromatography step and that was enough to get a sufficient level of purity of the product.

It's therefore a very potent tool, and especially because of the high capacity you can concentrate the product very efficiently in one single step, which is very convenient for AAV. Because as mentioned we have to concentrate the products so much to get the final dose, that it's a real benefit to have this chromatography approach.

MM: I agree - It's an invaluable tool and enables you to really quickly purify and obtain high purity vector. We not only use it for exploring process development but also for research production and all throughout the organization.

One thing we would want to see in the future is something that can be more high throughput. Something that could be more on a convective media such as a monolith or a membrane, because with viruses they are larger than traditional proteins, so you're not able to really maximize the efficiency of the whole chromatography bead with AAV, however if you're on a more convective media format you can actually unlock a lot more productivity potential.



“...often these constructs and molecules are being developed in research but the focus is mainly around the efficacy and safety of these vectors. There’s not much attention paid to the manufacturability of these vectors.” - **Meisam Bakhshayeshi**



MB: I agree with the other panellists in terms of affinity chromatography being a very powerful process to be able to achieve very high purity and high recovery in one step.

One thing I can add is around the cost of these affinity chromatography resins. Obviously these resins are costly, sometimes equivalent to almost half of the cost of raw material for your downstream process. So I imagine as the interest in these AAV gene therapies and using these affinity resins grow, we’ll see a reduction in the cost compared to what we’ve seen, similar to protein A in mAbs, over the past 30 or 40 years the cost has dropped significantly and now it’s a more economically efficient process.

OT: In terms of manufacturable, scalable processes, I think the affinity chromatography approach is a viable solution in terms of obtaining AAV viral vectors, with the different serotypes the field is working with and to a high degree of purity and yield.

It’s been proven to be a scalable platform for the mAb world, and I think the same could apply here as well.

With this affinity chromatography platform, we set out to combine the high selectivity for different AAV serotypes through the Capture Select technology, with the Porous bead which immobilises those ligands, to get to that high throughput that Michael mentioned earlier. In terms of having high capacity, high selectivity, as well as high throughput affinity resins that would be platformable for a wide range of AAV serotypes.

Q What are some common pitfalls of what can arise in your process developments. What do you wish now that you’d known as you developed your own process?

MB: One thing that’s not necessarily specific to AAV and could be generalised to other therapeutic modalities as well, is often these constructs and molecules are being developed in research where the focus is mainly around the efficacy and safety of these vectors. There’s not much attention paid to their manufacturability. And I’m talking about the whole process in terms of how you can manufacture them in cell culture, what would be your cell culture titer? Are there any particular impurities that are difficult to remove that result in a very low yield downstream process? Or you might have some stability issues or formulation issues downstream.

Perhaps there is a need to have a more direct interaction between process development and research, very early on, to be able to do a pre-assessment of the manufacturability of these vectors. That way you could avoid a common situation whereby you have to work around the issues and limitations you have with regards to that particular construct.

MM: From my perspective, I really don’t see much of a difference between gene therapy and mAb process development – it all comes down to first principles. You have to develop a high quality process. You need to develop an in-depth understanding around the process, robustness and understanding your molecule.

If you don’t do that, no matter what modality you look into, you’re going to have problems in manufacturing. This is why at Homology we’ve absolutely invested a lot into our process development and manufacturing capability to develop this, so we de-risk this going into manufacturing. Because we don’t want headaches when we get into manufacturing. We want to deliver. And so far it’s been paying off for us, where we have great understanding of our upstream, downstream process and within the manufacturing we don’t have many issues.

The other thing, as Meisam mentioned, is centered around working with your researchers. That’s something we really do a lot here at Homology – we’re all one team. Working with research to give feedback on anything they’re working on, and processes we’re working on, and having that symbiotic relationship so at the end we’re making the best product to deliver to patients.

One thing in particular we’re investing a lot of time into is around plasmids. It’s one of the critical raw materials you can have for these therapies at the moment and we’re really working a lot with research on the understanding of plasmid design, how that impacts the product and process. We think by taking these approaches we’re going to end up improving our productivity, improving our yields, and ultimately developing high quality product for patients.

The more you open up to vendors, the more you work with them, the more you collaborate, that's how you're going to drive a lot of innovation. It's really a symbiotic relationship." - Michael Mercaldi

OT: We support customers out in the field and one recurring issue we hear is that of recovery and that can depend on the different serotypes that customers are working with. I think it comes back to what Mike was saying, that the work needs to be put in, in process development to understand your molecule and design the process that will drive it to optimize recovery or any other issues that could be there. That's a thread I hear in terms of recovery.

MH: I think the common pitfalls we face in the gene therapy field and especially AAV is the fact that usually at the research level the vectors are produced using research methods like sodium chloride centrifugation as a purification step. This tends to hide the issues that could appear as you develop your processes for clinical manufacture and in particular scale up.

I have seen in the past some issues during scale up, such as decrease in productivity for transfection processes for example, or lack of consistency in the product potency using a baculovirus process.

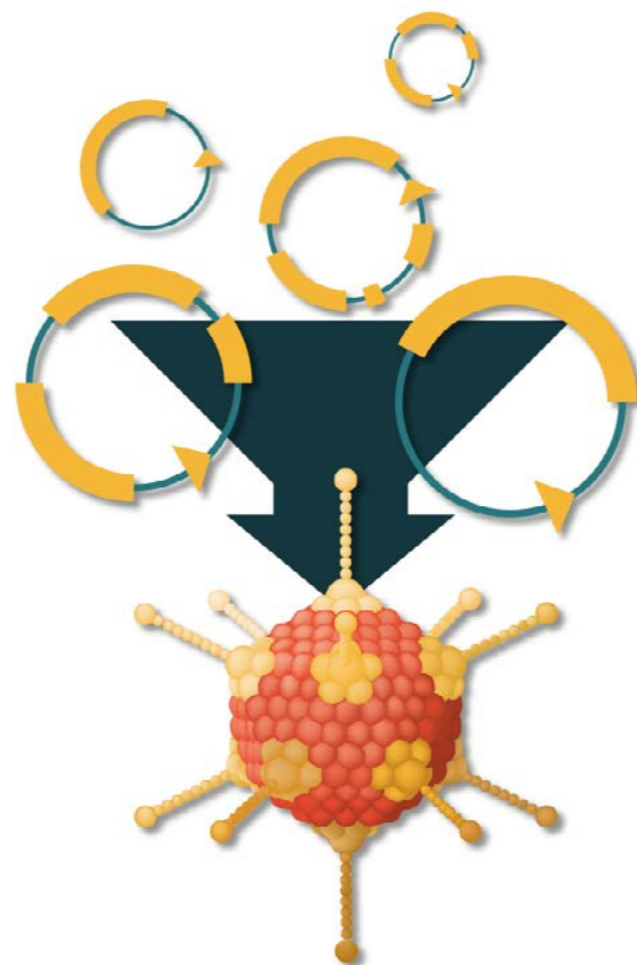
These kind of things tend to appear late in development, during scale up. Therefore I tend to recommend having a process which is closely resembling the GMP process at small scale, with generic material for studies and to fully characterise these vectors at an early stage.

MM: Just to add on to that - you really need to be working on this molecule before you even get it to the process development stage so you can really set it up

for success. Therefore, having that understanding, not only of the process but molecule understanding, it's really going to set you up in the long run for successful process development and eventual manufacture.

OT: We are constantly hearing the challenge around the separation or good resolution between empty and full particles. It's something we think about here - it would be great to have a platformable purification solution around the polishing, whether it's anion exchange, surface chemistry, or hydrophobic interaction, just different selectivities that can enable the resolution of empty and full particles.

This should also help in terms of the recovery challenges that we hear as well. This is why our emphasis has been on establishing a single purification platform for affinity capture, that addresses multiple AAV serotypes but also ultimately having a single purification platform around the polishing to obtain the full capsid enrichment.



Q How important is the analytical toolkit when you're developing your processes, and does it meet your current needs?

MM: For analytics, that's really the eyes and ears of the process. Without good quality analytics we can't develop our processes well and ensure product quality and patient safety in the long run.

We've developed a whole panel of assays here that can ensure product quality and patient safety, which we're using in our manufacturing process right now. One of the big challenges for the field is being able to supply your analytical team with a lot of high quality vector that's required in order for them to develop the assays they need.

It also comes back to what I was saying about process understanding. The analytics team is really invaluable to develop product understanding. There's a lot of assays you would perform that may not be what you're going to release with the manufacturing, but those things give you a lot of deep understanding that you need in order to improve overall productivity and ensure you're developing a better product for patients at the end.

MB: I agree with Michael that analytics is an important part of process development - without it we can't develop robust processes. Just as we have talked about the gaps and challenges we have in process development, there also exist gaps in analytics. One example concerns the variability of some of these assays, which makes it difficult to develop a very robust process as the ranges in your process parameters you get

from your assays can be highly variable. So precision is very important.

The other pain point is really around the throughput of these assays to support process development. A lot of these assays are cell based and therefore have a very long turnaround time. So there is definitely room for improvement and this could be achieved by automating these assays or using robotics to miniaturise assays.

OT: Certainly analytics is an area we have invested time in recently, to develop new tools in this space. Specifically around vector genome titration as well as full and empty determination. And specifically to address some of the challenges that were mentioned in terms of variability, enabling better control around variability, accuracy and precision of these assays, as well as meeting the high throughput requirements that were mentioned earlier.

MH: I totally agree with the previous comments, about assay variability because it's a nightmare to develop downstream process when your variability can be up to a factor of two. I would add that a proper potency assay is a big challenge with AAV vectors. And it's a tool that usually arrives quite late in the product development.

Q Something we hear quite a lot of talk around is sometimes a perceived lack of clarity around what's required from the regulatory agencies when it comes to viral vector manufacture. It would be great to hear any insight if the panel can share their experiences, and whether they feel there is sufficient clarity?

MB: In terms of regulatory guidelines, obviously we are not quite at the stage of protein biologics - there's a lack of clarity and information/guidelines specific to gene therapy. Having said that there were some guidelines that came out in July 2018 and one in particular around CMC for AAV manufacturing, which was very detailed and informative in terms of what a company needs to put into IND filings and applications.

What's missing, as more gene therapy and AAV products move into late-stage characterization, is more guidance for BLA applications and registration applications. Obviously at that stage the bar is much higher, so there's a need for more guidance and clarity.

This is a new field and that's the same for the regulators as well. So we need to work very closely with them trying to help them understand where the challenges lie and where the gaps are in the manufacture of these viral vectors. That will help define a lot of these guidelines that can be used in the future for manufacturing of viral vectors.

MM: The FDA draft guidance that came out last year we thought was very informative, and we felt was very sufficient and gave us a clear roadmap for our IND filing – which was approved earlier this year.

As Meisam said, looking forward to later stages, ICHQ8 and ICHQ11, they're geared towards small molecules, antibodies and everything else. It would be interesting to work with the regulators and understand if those guidances are relevant for what we're doing, and if so, what is the expectation as we move towards the BLA stage. Because only one company has walked that path so far, the regulators are also learning, in terms of what we want and how they can work with the innovator companies to ensure we're delivering high quality products.

MH: From an EU perspective we can find there is a lack of clarity on direction in the guidelines. But actually this lack of clarity is there on purpose to provide more flexibility to therapy developers to create their own methods and specifications related to their product.

The only thing I would say is when filing your very first IND is that it's very difficult to see where you stand compared to other people who are also developing vectors. Because this kind of information of course is not public. I think it would be very good if we could have more communication between companies to see what is the maximum level of purity you can achieve, what is the acceptable level of empty capsids we could have in a product for example. That would be very interesting and not necessarily something we could demand from the agencies, but more a kind of information sharing within the industry.

Q What further improvements or innovation would you really like to see in AAV or viral vector manufacturing in the near future?

MM: For us, it's about making more tools that are geared towards the protein nanoparticle separations or processing, which is basically what a virus is. Convective separation media is somewhere we can really work with the vendors to improve this component and reduce our overall processing volumes.

Working with more of the filter consumable vendors to develop filters and consumables that are smaller volume and less absorptive will also help enable more high throughput manufacturing.

Finally I think smaller volume GMP processing systems would be high on my wish list. A lot of our current systems are geared towards the antibody market and I think having

access to some systems that are more geared towards the AAV market would be really advantageous to us in the long run.

MH: For upstream process, we need to decrease the cost of goods and one area in particular would be transient transfection where the plasmids and culture medium are very expensive. Ideally a stable cell line would be fantastic for continuous and large-scale production of AAV.

For downstream process, I think an industrial-friendly method to enrich the full capsids would be great. Currently people mainly use ultra centrifugation which is not very industrial, and chromatography may lack consistency, I think perhaps because also conditions vary from one serotype to another and one product to another.

MB: As Michael mentioned, a lot of the technologies we are currently utilizing were really developed and optimized for protein biologics, and we're using them to purify AAVs. So there's definitely some gaps in terms of having access to the right scale-down model for these unit operations for filtration and chromatography; And especially as we move towards cross-characterization, that would be critical.

Having access to enough material to perform development and again as we move towards characterization, that's key. The challenge and bottleneck is low cell culture titers. Improvement in culture titer would be very important to support these activities.

And we talked about assays - having access to more advanced analytics in terms of accuracy, precision, and triplets, that would be key to support the future process downstream problems.

OT: I want to thank the panel for the feedback they've provided, because as vendors we have responsibility to understand the future needs of this sector so that developers have the tools they need to manufacture at large scale.

Certainly that was our thought process around affinity chromatography, we wanted to enable the gene therapy field to obtain purification of the AAV viral vectors, with the wide range of serotypes, in one step, at high purity and yield, through the Porous Capture Select AAV resins with high specificity and capacity.

However, there is still a lot of work to be done and we are looking at improving throughput to make sure that the tools are effective when large scale production is occurring. In addition, full capsid enrichment is also something we are looking at - whether we can provide a platformable solution as Matthias mentioned, so we can solve some of these challenges together in collaboration with our gene therapy customers.

MB: One thing I also wanted to highlight is that as we talk about some of the gaps and challenges that exist in process development, similar knowledge and experience gaps exists at contract manufacturing organization (CMOs). A lot of gene therapy developers outsource their manufacturing to CMOs and as this is a very new field, there's a lack of experience and knowledge with regards to the manufacturing of these AAVs; particularly around using single use

manufacturing. So that's an area that can be improved in the future and undoubtedly as this field grows, more manufacturing of AAV will happen, and that knowledge and experience gap will shrink.

MM: At Homology, we're taking a balanced approach: doing external and internal manufacturing. We just opened up our internal manufacturing facility where we're developing a lot of that manufacturing knowledge that you just won't get unless you're actually the person in the suites and handling the equipment and producing these vectors. Because that's actually the stuff that's important at the end.

We can make the most elegant process, but if it's not going to work in manufacturing, it's not going to work, period. So having that kind of relationship where we can work closely with the CMOs and have internal manufacturing capabilities ourselves to give feedback to the process developers, that's really going to advance the field.

MH: I agree with what has been said and that not all CMOs can provide very deep support in terms of process development and vector knowledge. And therefore our strategy at LogicBio has also been to develop our own internal capabilities for process development and analytical development to be sure we can bring this support to the CMO.



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