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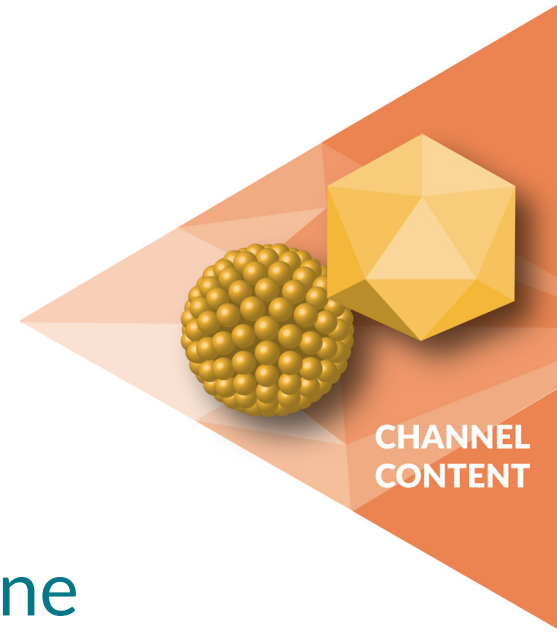
VECTOR CHARACTERIZATION & ANALYTICS



INNOVATOR INSIGHT

COVID-19 mRNA vaccine approvals: key lessons for cell & gene therapy and mRNA therapeutic development

Joseph Barberio, Christoph Kröner, Venkata Indurthi & Scott Zobbi

In this *Cell and Gene Therapy Insights* Expert Roundtable, our panel of four experts will answer two central questions for novel biotherapeutic developers: what can the cell and gene therapy field learn from the prophylactic vaccine approvals? And how will the vaccine's success help accelerate the progress of mRNA therapeutics?

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Q & A

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Q What would you pick out as the key development challenges facing mRNA therapeutics today?

SZ: From my perspective as a vendor, a lot of the challenges come back to the fact that developers like Joseph and Christoph don't have the purpose-built tools they need to get the job done. A lot of the tools that are being deployed in this space are legacy products from mAbs or protein therapeutic production; they work, but they may not be optimized. There's a large development challenge around that, and as the space becomes larger and more invested, you're going to see a lot more purpose-built solutions.

The other thing that I think will be a theme throughout today's discussion is the supply chain. mAbs have been around for 40+ years and have a well-worn supply chain, whereas mRNA therapy has only existed in this iteration in the last year so there are huge gaps within supply chain that are currently getting built out.

VI: I agree with Scott about the supply chain. As the field has exploded over the past year, the demand for raw materials has become very high and there are still only a few companies on the market to ensure supply chain for all processes. Plus, it's not completely clear or defined what quality is needed for which material. There is still a lot of space for development.

JB: With regard to analytics, we need some regulatory guidance to clarify what we're aiming for – the quality of the process as well as what the analytics can tell you and the current state of the analytics.

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There is a lack of experienced CMOs out there, and those that exist are under stress due to demand for mRNA in the biological landscape. The lack of an experienced talent pool for growing biotech companies is also a challenge, making it hard to fill out those positions.

VI: There are challenges in both upstream and downstream. We see certain developmental challenges upstream, right from the enzymes, because most of the enzymes currently are wild types, which have certain disadvantages. The more you can fix upstream, the less pressure you put on downstream.

SZ: Jo, Venkata, Christoph – do you think there is an assumption that we have all the technical challenges solved, when in fact there are a lot of unanswered questions?

VI: Definitely. Just taking the topic of quality level, we are told 'the best available quality level' but that can mean different things.

Even as far as technology goes, there are multiple approaches to get to your end product; there is not just one way to do things. There is no question the field is in its ascendency, but because of the accelerated timeline, there's a lot of information to process and learn in a very short period.

SZ: There has been a lot of pressure in this past year and has accelerated the platform. The level of development, the level of focus, and the amount of funding that has gone into this market are like nothing I have ever seen before.

Everyone's backs were against the wall on this, but I got my mRNA vaccine last week and I wouldn't have put that in my arm if I had any doubts about it. I feel it was produced with the highest level of quality and efficacy available, and I'm glad that technology existed at the right time for this too.

JB: It was interesting how mRNA therapeutics paced the field, whereby the sequence was made known to all different companies at the same time, and all different modalities, and two came out clearly on top.

Q In addition to the influx of funding to the mRNA space, how else has the picture changed since the successful development of COVID-19 vaccines, and how might that alleviate or add to some of the bottlenecks?

VI: The COVID-19 vaccine has accelerated the platform by about 10 years and changed the picture for RNA completely. Now people understand the potential of RNA, more and more people in the space want to promote more and more tools, but that would add to some of the challenges that we've just discussed, such as a shortage of raw materials. Alternatively, I see mRNA being one of the most revolutionary technologies in vaccine and therapeutic spaces.

CK: Now we have approved products, we have a clearer – albeit still developing – picture of the quality level we need to reach for the product.

SZ: I'm looking forward to people paying more attention to mRNA as a therapeutic. You're going to see ideas percolate to the surface – things we've never even thought of before.

JB: One of the biggest gains from the vaccine approvals is establishing trust in this modality from the public and investors. With the efficacy of these two vaccines, there will certainly be more investment in the space – both in the tools and in the biotechs themselves.

Q You all touched on the challenges around the downstream processing side. What are the specific bottlenecks around mRNA downstream processing, and could you point to any recent innovations in this area that you feel are helping to improve or ensure product quality and safety?

CK: We have large molecules with a lot of negative charges so purifying RNA from non-functional RNA or DNA is a real challenge. We have made some large improvements during the purification itself to get the pure product and to achieve upscaling.

BioNTech and Moderna both have large programs focusing on individualized cancer therapeutics, and in both, we had manufactured a lot of mRNA batches for GMP (around 1000 here at BioNTech). That gave us a lot of experience of how to manufacture mRNA in multiple batches quickly and achieve key conditions, which helped a lot.

VI: Downstream purification is a challenge. We've been using tools that were not designed for nucleic acids, leading to lower binding capacity and having to do multiple lots to get to the level of purity needed.

SZ: It's important to take a holistic view of the process. We often see customers focusing on how to solve a downstream problem, but it turns out to be an upstream problem. For example, the titers are very low, the product quality isn't there, or you're trying to remove a reagent or contaminant that wouldn't necessarily be present if you optimized your upstream.

Picking up on Christoph's point, scalability is also a major issue. One of the worries that I have is that a lot of customers will be moving into the space with a very academic or R&D mindset, and they're going to choose solutions that are not scalable and are unsuitable for GMP manufacturing.

JB: I would just reiterate that the binding capacity of resins and the throughput and mass challenges to TFF membranes are low compared to what you see with other modalities.

SZ: As Venkata mentioned, there is no one right way to do this; it's going to be different for different constructs, and different manufacturing scales.

VI: That is a good point, and I'll add a CDMO perspective to that. Often, CDMOs don't control the design of the RNA, and a lot of purification methods are dependent on the

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secondary structure sometimes. That is challenging for us because we see multiple designs with multiple final specification requirements.

Q What more could be done upstream to further alleviate these downstream issues you've mentioned?

JB: The control of process inputs is very important. You must have a deep process understanding and characterization, as well as robust associated analytics to understand how both upstream and downstream iterative process development is affecting the product. You need to ensure high-quality raw materials and starting materials and understand the impacts of those impurities on the profile of the drug product or drug substance.

In my view, that is the most important thing on the upstream side – understanding the inputs you're putting in and how they impact things on the back end.

SZ: Absolutely. Having well-characterized reagents, and the right quality level of reagents (whether GMP or ISO) is of huge importance. Everything has happened so quickly that manufacturers are taking the highest-level quality they can get, but we're now looking to the regulatory agencies to give guidance on what's required in that space.

CK: The most important raw material that goes into the mRNA is the DNA, so as well as the level of quality needed, we need to know the level of sequence correctness that ultimately defines the product.

Q Something we've touched on in this discussion is retrofitting technology and platforms from the mAb space to meet urgent needs in mRNA manufacturing. What enabling technology innovation is needed to help address these bottlenecks we've discussed?

SZ: I'm sure there are a lot of enabling technologies out there just waiting to be discovered. For one thing, I'm convinced we're going to start to see more and better-modified enzymes. I believe that in the mRNA space, we are going to find or modify enzymes to improve yields, transcriptions, and capping that has yet to be discovered or understood.

I also think there is going to be a lot more work focused on polish chromatography. There are different modes of chromatography you can use to purify mRNA, and looking at what the key contaminants are and how to polish those away, whether it be unreacted NCPs or residual enzymes, or double-stranded RNA, will be an important area in the future.

JB: I would add that, to understand what needs to be removed, we need analytics. As a process development person, I would say analytics are almost more important than the process development work itself because if you don't know how to quantify what's happening and understand the effect on the product, that work is useless. The ability to find good functional potency assays or predictive assays, to have predictive models, to minimize

the animal studies are all important. The field does need potency assays to determine efficacy as there is a great deal of difference between *in vitro* and *in vivo* processes when it comes to mRNA.

So it's a priority to work on some high-quality analytics, and have novel approaches to performing functional or potency assays, to minimize the amount of work that needs to be done in the animal studies.

CK: There's a lot of analytical knowledge we can take from diagnostics – but we need to find a way forward to introduce these complex technologies to the pharma world.

Q How could machine learning contribute to the development or production of mRNA therapies?

JB: I would say it is certainly applicable and is currently being implemented at some of the newer startup biotechs. I would expect that it's probably being used in some of the larger mRNA companies as well.

VI: it is a very powerful tool that can be applied in several ways, whether to improve your raw materials, or to understand RNA structure, design, and so on.

CK: I agree machine learning is an important future direction, but the molecule and the reaction itself is so complex and depends on so many parameters that currently there is no straightforward way for us to put the data into the machine and find the perfect mRNA or the perfect process to manufacture it.

JB: You need to understand the entire process. And the entire folding structure of the molecule and how each impurity can affect that, as well as the kinetics of the reaction, to understand exactly what your product needs to be.

There needs to be a better understanding of the important characteristics from sequence all the way to structure, around mRNA as a therapeutic modality, before machine learning can truly be trusted to move forward a platform, as opposed to empirical data and design of experiments.

VI: Initially I think we need to look at applying machine learning in modules, for one particular component in the entire process, rather than holistically.

Q Raw materials came up earlier in our discussion. What specific issues have you encountered and how have you sought to address them?

VI: Extremely long lead time for raw materials is one of the biggest issues in the field at present. There are raw material shortages across the board, and we are starting to see huge enzyme shortages. I do not have a clear answer yet on how we can address that; we are working through it right now.

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SZ: Again, it comes down to the speed the at which field is moving. A year ago, there were no approved mRNA therapies; this year there are two approved mRNA therapies with commitment for billions of doses. The industry is having to build supply chains from scratch for a majority of the reagents, lipids, and raw materials needed. There is a huge investment going on right now to build out that supply chain, but it still takes time.

I find it frustrating when you hear people saying "if BioNTech or Moderna just shared their sequence and their information we could be producing million-dose batches tomorrow." My answer would be, with what? Even if you knew how to make it, there are no reagents, no enzymes, no NTPs available. That's why I think the focus needs to be on the key vendors who already have the infrastructure in place, like BioNTech, Pfizer, Moderna, CureVac.

JB: This would be a supply chain issue for any modality. It's hard to think of a time when the patient population has been, essentially, the entire world. It's not just enzyme shortages, supplies of every kind are stretched, from pipette tips, to bags, to conical tubes. There are queues in CMOs for production, queues in outsourced analytical development organizations.

Q What do you feel are the key lessons that mRNA vaccine and therapeutics makers could learn from each other?

JB: I think we've learned that mRNA-based drugs can be quickly scaled up to make very consistent products. And mRNA is now a proven, safe, and efficacious modality for drug delivery. There are massive datasets that coming out of the vaccine programs, involving hundreds of thousands of doses in all sorts of patients, which will be invaluable to those developing mRNA therapeutics. Once tissue-specific delivery is solved, the sky is the limit for the mRNA space.

SZ: Joe mentioned tissue-specific targeting, and a lot of the work that needs to happen next is not just with the mRNA itself but on the delivery mechanism. Is a liquid nanoparticle really the right way to go? Is it good for certain things but not for others? There are so many novel packaging mechanisms that are being looked at now or have the potential to move forward. There's a lot of excitement in that space.

Q We've had lots of questions from the audience on analytics. What do you see as the biggest challenge in mRNA analytics?

CK: That is a question we are asked more and more often. And it's topical because it is one of the main challenges that we face – mRNA is a large molecule with a complex secondary structure. Having the mRNA as a full-length homogeneous configuration is the aim, but that's not what we get after *in vitro* transcription.

For example, *in vitro* transcription can produce shorter, double-stranded mRNAs. Acquiring knowledge about this completely heterogeneous population of mRNA is very important. In the future, I believe we need to go down to single-molecule analysis of the mRNA.

Q The panel has mentioned that mRNA characterization, particularly folding and forms, is a crucial aspect for downstream processing that needs to be better understood. Could you elaborate on this aspect?

JB: When it comes to the purification and impurity profile, everything matters. Plasmid quality is important, that's your template for the starting material, and different IVT conditions can potentially create different types of impurities, so understanding how those impurities are affecting your downstream purification, or the integrity of the intended full-length product, is important. There are certainly levers that can be pulled that make a higher quality product than others, and you must understand what those are.

The biggest difference between the bench scale and the high-quality commercial manufacturing is the analytics. You don't know you have impurities in the material unless you check for it with high-quality analytics. Bench-scale, silico-purified material looks the same as high-quality multiple chromatographic purified material if you look at it with rudimentary analytics.

Q Do you feel that the BioNTech and Moderna mRNA manufacturing processes and in-process analytics will become the regulatory standard, or will further regulatory scrutiny be in place once the pandemic pressure is removed?

VI: It will be a standard for now, but once the pandemic is over there will be more and more scrutiny. What regulatory agencies are looking for will evolve as the technology evolves, whether from a process impurity standpoint or product impurity standpoint.

JB: I would reply that BioNTech and Moderna have fairly mature processes. They have been working on these technologies for quite some time. I don't think there were shortcuts in the release testing and analytics and qualification of the analytics. So I think there might be a new benchmark in analytics that has been established, but I don't think it will necessarily change the amount of scrutiny on release-testing protocols, although the speed at which everything is reviewed may decrease post pandemic. But I would hesitate to suggest that the release panel wasn't of the highest quality for the approved vaccines.

CK: I think it's a good benchmark, but there are opportunities to improve that. And we will have that opportunity because the situation in the future will be different. I hope we will never again face such high demand in such a short timeframe.

Q What does the future hold for mRNA, and oligonucleotides in general, and how and where will they be deployed next?

CK: We're still at the beginning with mRNA, and there are so many different approaches to use that technology and so many different opportunities.

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JB: In my opinion, it's going to be deployed in almost every setting, unless you need gene addition. We've already seen vaccines for infectious disease, and cancer vaccines will follow. There are companies out there that are using replicating mRNA, and cell-type specific expression using logic circuits. There are the CRISPR tools for base editing and prime editing. We're just scratching the surface with the vaccines. As the supply chains grow and money comes into the space, mRNA will become one of the core modalities for fighting all diseases.

VI: We are already seeing that in the CDMO space, with several different applications, such as protein replacement therapy, coming through. The technology is already accelerating quickly.

SZ: This is an incredibly exciting time and I'm looking forward to seeing the new and novel ways that mRNA is used in the market to cure disease and treat patients. I think everyone here and listening would agree that's why we are all in this business – because we want to help society.

BIOGRAPHIES

Joseph Barberio
Director, mRNA Process Development, Strand Therapeutics

Joseph Barberio is a biochemist and molecular biologist with a proven track record of solving complex problems with innovative solutions. He is the Director of mRNA Process Development at Strand Therapeutics and oversees Strand's manufacturing strategy to support clinical development. With over fifteen years of industry experience, Joe specializes in process and analytical development and has extensive expertise in manufacturing of both viral and RNA based gene therapy medicinal products. The majority of Joseph's career has been focused on building platforms for small biotech organizations. Most recently at bluebird bio, he constructed and led the mRNA process development team, a group designed to enable gene editing programs. Earlier in his career, Joe held process development roles at Moderna, Percivia, and Acceleron Pharma. In addition to his work at Strand, Joseph also serves on the Board of Directors of Sophie's Hope Foundation, a non-profit charity supporting research for glycogen storage disease type 1b (GSD1b).

Christoph Kröner
Director DNA Process Development & Cap Technology, BioNTech SE

Christoph Kröner works as Director DNA Process Development & Cap Technology at BioNTech RNA Pharmaceuticals GmbH with strong focus on developmental work for BioNTech's various clinical projects using mRNA as drug substance. He has more than 10 years of experience working with nucleic acids like mRNAs. His work focused on mRNA-based therapeutics when he joined BioNTech in 2014 as a scientist. Christoph Kröner holds a diploma and PhD in chemistry from the University of Stuttgart, Germany.

Venkata Indurthi
Vice President Research and Development, Aldevron

Venkata SK Indurthi, PhD, is the Vice President of research and development at Aldevron (est. 1998), a biologicals CDMO with sites in Fargo ND and Madison WI. He received his

bachelor's in engineering in biotechnology from SRM university (Chennai, India) and his PhD in Pharmaceutical Sciences from North Dakota State University (Fargo, ND). After completing graduate school, he joined Aldevron as an assay development scientist and was focused developing assay for GMP release for biologics (DNA, RNA and protein). He then transitioned to a role of senior scientist, Product and Process Design (PPD) where he led the development of Aldervon's mRNA process and platform and more recently as the Director of R&D where he established all the R&D efforts for Aldevron. Dr. Indurthi leads efforts that to develop new platforms and innovative manufacturing processes for the company including (not limiting to) the mRNA platform, plasmid DNA, gene synthesis and cell free synthetic DNA platform. His team also focuses on the development of new products for the company such as enzymes with improved attributes (increased yield, stability or activity). Dr. Indurthi's research interests focus on the development of biologics. Particularly, new platform development from idea conception to commercialization. He has developed processes that are currently used for GMP manufacturing. Dr. Indurthi is also the operational head for the Aldevron RNA services.

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Scott Zobbi is a Senior Manager of Business Development for Custom POROS Resins within the Bioproduction Division at Thermo Fisher Scientific. Scott has a B.S. in Biology from University of Connecticut and an MBA from the University of Massachusetts. Scott has worked on chromatography applications in the biotech industry for decades with 22 years spent at Thermo Fisher Scientific. His expertise is finding solutions to complex separation challenges draws on his experience in cGMP manufacturing, process development, customer training, sales and product management. In his current role, Scott is responsible for managing globally the Custom POROS Resin program, including working with customers to identify needs, in-house R&D to develop solutions, and POROS manufacturing to commercialize bioprocess resins for GMP applications.

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