CELL & GENE THERAPY INSIGHTS

PODCAST INTERVIEW with:

Jeffrey Hung, General Manager, Vigene Biosciences, a Charles River Company; **Daniel Smith,** Executive Director, Global Cell and Gene Therapy Portfolio, Cobra Biologics, a Charles River Company; and **Horst Ruppach,** Executive Director, Scientific and Portfolio, Global Biologics, Charles River



Key topics in advanced therapy manufacturing: quality, safety, and supply

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Three experts in advanced therapy manufacturing discuss the challenges – and opportunities – facing cell and gene therapy today, including intensifying viral vector processing, strengthening supply chains, and navigating the ever-changing regulatory landscape.

Q As evidenced by recent meetings such as that conducted by the FDA's Cellular, Tissue, and Gene Therapies Advisory Committee, the safety of viral vector-based gene therapies is firmly in the regulatory spotlight at present – how are specialist CDMOs such as Vigene helping to address this key issue?

JH: On the organizational level, there are three lines of work we are following right now. The first is molecular gene therapy design. As you know, a gene therapy is only as good as the gene therapy on the plasmid, and later packaged into the viral vectors. As a development company for viral vectors, we have seen a lot of gene therapies that are not stable, causing the batch-to-batch and lot-to-lot variability, because the design was not right. I would like to see the industry standardize and be better at making stable and consistent gene therapy molecular design.

The second line of work is process development. We have often seen cases where the development process design was not optimal for gene therapy production. We have to rework a lot of processes we receive to make the process more robust and reduce impurities to a level that is safe for patients. That is critical, and we have done a lot of work on the process optimization and process development on our side.

The third line of work we have been doing is implementing best practices in the operation of gene therapy manufacturing with quality and safety. That is the last mile to the patient, so we need to implement good design and execute it flawlessly.

Q What is the latest progress in enabling viral vector process intensification, and where is further work required?

JH: Vigene was founded with the vision to make gene therapy affordable, so process intensification (scale-up) is core to our mission. I would like to highlight three aspects of how we achieve that goal.

The first is upstream process intensification. For example, we have been working on cell line development. The viral vector cannot amplify cells by itself for safety reasons, so all recombinant viral vectors have to be packaged artificially in cell lines. These cell lines differ dramatically from one another in signs of productivity and stability, so optimizing the cell line is important.

Second is bioprocessing intensification, including perfusion, is critical. If we can increase the yield of cells by a factor of two or four, the yield of viral vector will increase accordingly.

Third is downstream optimization, relying on advances in material science for downstream columns and membranes. Right now, we are partnering with several suppliers and partners to develop and verify those new downstream technologies.

Q What would be your advice to gene therapy developers struggling with the requirement for earlier process-related decision-making brought about by reducing development timeframes?

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JH: I have three pieces of advice, all centered around quality by design. The first is quality by molecular design – how to structure the promoter, how to structure the stuffer, and how to design the plasmid such that undesired packaging will be minimized. All of these are determined by the molecular design of the plasmid and gene therapy itself.

The second is the quality by process design. Manufacturing can only be as good as the process development and process itself. In other words, the safety, purity, and potency can only be as good as the process we develop. That means a lot of things need to be built in to consider maximizing potency and minimizing impurity.

The third is quality by material design. A lot of academics and gene therapy designers are working with materials, especially critical supplies, that are not GMP ready. When it comes to gene therapy manufacturing, we then have to go back to the drawing board and re-do all the materials supply and design. That takes a lot of time and brings a lot of risk to the gene therapy program.

DS: I completely agree with Jeff on all of that. From a development point of view, it's also very useful to help developers to think about the ultimate quality attribute they require from their viral vector, early on in that development lifecycle and how best to achieve that. What dosages are they looking for? What's their population size of indication they need to go after? We try to help them map out early on not just how much to make to support the patient population but how much is required for analytical development, qualification of assays, stability-indicating assays, so they have a clear roadmap of how much material is required for the development phases, early clinical phases, and late clinical phases before they get to commercial realization.

Given the speed that some of these products move through the clinical phases (for example, after being granted orphan indication and breakthrough status with the FDA) you may not have a lot of time as a manufacturer to change the processes between phases. Therefore, the process they start with at early phase must be the process they end up with at commercial phase. We want to help people make the right decisions at the front end because it's a lot of time and cost if you get it wrong as you move forward through the different phases.

HR: I would add that GMP aspects should be considered very early – in the preclinical phase, maybe even earlier. Even if you set up assays, those assays may be fit for purpose but not fit for GMP, and switching the assay, method, or equipment can cause major delays when moving through clinical phases.

Here at Charles River, we have the GMP background and the preclinical background to know what needs to be covered at a very early stage and smooth the path from the clinic to commercial. That's one of the strengths we can offer to our clients.

Q Ensuring a sufficient supply of high-quality plasmid has been identified as a key potential bottleneck for the cell and gene therapy field moving forward. How is Charles River positioning to address that?

DS: To put it bluntly, everything starts with plasmid. Currently, most viral vectors are made through transient transfection using a combination of 3–5 different plasmids to make

the viral vector. Therefore, it's really important to make sure the supply chain for plasmid is robust.

So how are we doing that at Charles River? Different developers can access plasmid at different grades through their development lifecycle. First. There is research use-only grade plasmid, and acquisition of Vigene and Cobra Biologics allows Charles River to make research-grade plasmid very quickly.

The next grade of plasmid is what we would call high-quality grade plasmid or GMP-ready plasmid. Again, Cobra and Vigene can supply plasmids of sufficient quality from both in terms of absolute functionality of those plasmids but also, within a regulatory environment, to allow traceability and confidence that the analytical support around it is in place. Documentation allows customers and potential developers to use that type of plasmid to file the right regulatory framework to support their clinical trial applications. As we move on in the development phase from Phase 1 through to Phase 2 and commercial, again GMP grade plasmid is important to be able to again ensure a level of quality and compliance for the product.

Charles River, through the acquisitions of Cobra and Vigene, now has a strong network of service offerings, in the US and Europe, to allow customers and clients to go from research-grade through to high-quality grade and GMP-grade plasmid.

There is a bottleneck in the industry for plasmid supply; however, by streamlining and harmonizing some of the service offerings across the CDMO network within Charles River, we're able to allow customers to access whatever plasmids they want, whenever they want, at whatever grade they want. We are ensuring that we build the appropriate capacity for either larger scale-up or larger scale-out at those different grades as the industry demands it.

Q How do regulatory and scientific requirements for plasmid differ for different products, for example, lipid nanoparticles, adeno-associated virus, or lentivirus?

DS: Again, I think it's interesting to dive into the different types of regulation around the use of plasmids and what plasmids can be used for. We've talked about viral vectors and how plasmids can be used transiently to support the production of viral vectors.

When you think about what the regulations are there for, it's to ensure patient safety from the point of view of clinical trials. The plasmids themselves are never going to be the product. They are there as critical starting materials to feed into vector production and, in the case of lentivirus, to make lentivirus that then goes on to potentially transduce a

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human cell for a cell therapy-based product – there are degrees of separation between the plasmid and the patient.

The regulatory environment is evolving. Traditionally, people have adopted the same approach for plasmids as starting material as we would apply for plasmids for direct clinical use. That means a lot of platforms, specifications, and analytical methodology have been built around the need to ensure patient safety from a clinical point of view. However, I think the industry is now starting to challenge that paradigm and assess what is really important from a regulatory and a specification point of view to make plasmid for critical starting material.

A case in point here is an mRNA sequence within a lipid nanoparticle. When it comes to the plasmid that was used to make the mRNA, is it more important that we remove all the residuals from it from host–cell protein, host–cell DNA, and host–cell RNA, or is it more important that the sequence is absolutely correct? It's an ongoing discussion.

The FDA and EMA have both recently announced new guidelines for the production of plasmids as critical starting materials – I would like to see more harmonization between those two sets of guidelines. As part of the CDMO network within Charles River, we need to understand how to apply the guidelines appositely across our network to give customers confidence that the plasmids we make for them are fit for purpose, both from a safety perspective and a utility perspective.

HR: I have a question for Daniel. Lentiviral vectors are also a critical ancillary or raw material because they are not typically given directly to patients but used as a material to transduce cells. However, the FDA advises that retroviral vectors should be considered like drug substances. Is that the same or similar with plasmid?

DS: To a certain extent it is. The recent guidelines have three main areas of compliance. Full GMP, non-GMP, and within the 'principles of GMP', which I would call a gray area in the middle.

Plasmid that is made for transient transfection for viral vector falls under principles of GMP, as does a lentiviral vector used for modification of cells for cell therapy. Plasmid that is used to make mRNA also falls under principles of GMP but mRNA itself falls under full GMP because it is the clinical product.

However, I don't think people have really adopted this approach yet for lentiviral and retroviral vectors. There's still a lot of discussion around what is the absolute regulation around this. And I think the other thing to consider here is that the regulations are also linked to the phase that you're at.

Principles of GMP are very easy to apply for early-stage material. As you move through to late-stage, Phase 3, and ultimately commercial material, most quality systems from large pharmaceutical or biotech companies will insist you go to full GMP. I think it's a sliding scale between early-phase to late-phase, principles to full GMP. Horst and Jeff – it would be great to get your thoughts on this.

JH: I totally agree with you, Dan. We need to design the process such that it can be easily scaled to be GMP compliant. For instance, if we are talking about the master cell bank for *E. coli*, it's better to structure and make a full GMP compliant master cell bank to start with instead of a research-grade master cell bank to make the early-phase clinical trial plasmid and

later on the viral vector raw material. That's just one example of how we can be compliant and structure the program to be fully integrated with commercial readiness.

DS: We've talked about process, and we've talked about the different regulations and how people are applying those, but I think the analytical and characterization side is also important here and there is less flexibility in that space. To be able to release products under the principles of GMP, the analytical assays and methodologies need to be fully qualified, if not validated, for certain points. With a very strong baseline for analytics, it's difficult to characterize your product as well.

There has always been a phase-appropriate approach to analytical characterization and regulation – whereas for early-phase you might use fit-for-purpose assays, you might go on to use qualified assays, and only at late stage go for full validation of those assays. It's the same principle we're trying to apply here. I think it's really important to make sure your analytical characterization packages are, if not fit for purpose, at least properly qualified for principles of GMP work.

HR: These aspects are very important – we could fill an entire podcast talking about it so I'll just add that often our clients have a different understanding of qualification of an assay and phase-appropriate qualification. It's a challenge because you have compendial methods that are more or less easy to use, and various other assays, so we have a lot of discussion with clients about what is needed. It's a topic where clients have a lot of confusion in terms of what the regulations mean for their specific case. And the answer frequently, unfortunately, differs case by case.

Q What lessons have you picked up during the COVID-19 pandemic and response to it?

DS: It's been an interesting 18 months. I've been privileged to work in an organization, Cobra Biologics, that has been at the forefront of the production of viral vector vaccines for the pandemic, DNA-based vaccines for the pandemic, and DNA raw materials to support mRNA vaccines for the pandemic.

What have I learned through that? That if you all work cooperatively and collaboratively, you can achieve a lot very quickly. It takes a common purpose to be able to move things rapidly through development, scale-up, and into GMP environments, with the right level of qualification, validation, and compliance, and with a real foresight on how to move this as quickly as possible without compromising on patient safety or cutting any corners. Working with a common purpose and in collaboration, we have been creative and challenged our normal paradigms of working.

We have also had to learn to ensure robustness and resilience in some of our workflows, and I think the most important thing is planning around this. The last 18 months have been a rollercoaster and what we're seeing now as a result of all of that is extended lead times and a lack of robustness in supply chains. Business continuity, good supply chain planning, and the ability to move things around quickly within the appropriate regulatory framework are

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essential to get things done. That has been a phenomenal challenge, and it's been a huge privilege to work with some really dedicated people to get things moving.

Q Can you give an overview of current regulatory standards or guidance for viral clearance in gene therapy manufacture?

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>
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HR: First, let's clarify the terminology. Viral clearance means the capacity of the product purification process to remove or inactivate adventitious viruses. As you can imagine, for vectors like AAV or lentivirus, the capacity to remove or inactivate viruses is limited, because the product itself is a virus particle. By contrast, for recombinant products, there are usually strong viral clearance capabilities. For the cell therapy area, you don't have any capabilities to remove or inactivate viruses in the production process.

When we talk about viral clearance, the ICHQ 5A guidance is most often referenced, even though the scope of this guideline does not address viral vectors. However, the ICHQ 5A is currently under revision and the scope will be expanded to include gene vector products.

The question most clients have is: do I need to apply it? Do I need to analyze the viral clearance capacity of the downstream process? Of course, if you go commercial you have to analyze. It's part of the validation of the manufacturing process before going commercial: the demonstration of the capacity of the downstream process to remove or inactivate viruses.

However, many questions come to us about early-stage. For example, a client may ask: what about if we want to step into clinical Phase 1, do we need to analyze viral clearance capacity at that stage?

This is a little bit confusing if you look at the regulation. For instance, the most recent FDA guidance, *"CMC Information for Human Gene Therapy IND Applications"*, does not request general viral clearance validation when you step into clinical Phase 1. However, there is one exception. If there is a viral contaminant, you should demonstrate, even at that early stage, the capacity of your downstream process to remove those viral contaminants. A known viral contaminant could be, for instance, a helper virus. If you use a helper virus in the manufacturing process, you need to demonstrate that this helper virus is inactivated or removed in the downstream process.

The same principle applies if you use a baculovirus system – you should demonstrate the removal of baculovirus or HSV if that modality is used. And for some production cell lines like the Sf9 insect cell line, there are reports that this cell line is contaminated with rhabdovirus, so it's a known contaminant. If this is confirmed, you must demonstrate the clearance of this virus as well at early phases.

There is also a European draft guidance for investigational ATMPs, and these are much clearer, saying the process and the viral removal inactivation steps are expected to be validated

prior to the first-in-human clinical trials. It may change, but right now the expectation in Europe is that you analyze the viral clearance capacity in general, independent of whether you have a relevant viral contaminant like adenovirus or not.

Q What would you pick out as the key recent advances in terms of the available assays and analytical tools, and what are some of the important considerations in employing them?

HR: Very important, especially in the cell therapy area, are rapid testing capabilities. There are already solutions available like for mycoplasma and sterility testing. Some assays can reduce the turnaround time for sterility testing down to 7 or even 3 days, whereas compendial methods take at least 14 days.

Another development I see is performing on-site testing instead of shipping materials to a CRO, especially for in-process testing and release testing. The above-mentioned sterility testing technologies are set up for ease and robust use – ideal for on-site testing. Rapid mycoplasma testing still requires PCR logistics and expertise. The next step is from on-site testing to online monitoring. I have seen online monitoring systems that are connected to the bioreactor and do deep analytics of the phenotype of cells. They are so sensitive that they can differentiate infected cells from non-infected cells. Those tools are also used to analyze the transfection process in the bioreactor because they can even differentiate transfected and non-transfected cells.

Another technology that I regard as highly important for the characterization of starting material, especially cell banks, is high-throughput sequencing technology (next-generation sequencing). This is a comprehensive tool that can be used for two aspects. One is to screen for pathogens that like mycoplasma or viruses. The potential of this technology is that it can find and identify any kind of contamination – even unknown contamination – because it sequences any nucleic acids that are in the sample. Another use is to genetically characterize cells. For instance, the copy number of vectors, off-target integrations, and identity of cell lines like iPSCs.

I regard next-generation sequencing technologies as the most important technology that we will see used in the future for the quality assurance of critical raw materials and products, whether it's gene vectors or cell therapy products.

The challenge is that next-generation sequencing is a complex technology. It requires processing of data like data filtering. There are many, many aspects that you must consider. And under GMP it's even more challenging. However, many groups are working on these challenges, including regulatory agencies like the FDA, who have built working groups to make it possible to use next-generation sequencing in a GMP environment.

Q CMC has certainly been in the spotlight of late with late-stage cell and gene therapy developers running into issues with the regulators – what would be your advice to early-stage developers seeking to prepare for an increasingly stringent regulatory environment?

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HR: In addition to the points already covered, I would add that taking care of data integrity is very important. Protection and traceability of data is an important demand and should be considered very early on in the development process.

As you develop your product, you should document what you do, and you should take care that the data you create is securely stored for use as justification for the next steps. If you have a clear and documented path of how you selected the assays, how you created data, that will help you later in moving forward and avoid delaying the process. Even though that data may not be under GMP, if it is well documented it will be appreciated by the regulators as supportive data to justify your approach when you are in clinical phases.

For example, there are specific guidelines for potency assays to demonstrate the functionality of the product, in an *in vivo* or *in vitro* assay. This can be a complex and time-consuming assay. If you use equipment for cell-based *in vitro* assays, make sure that this equipment is part 11 compliant, which means it fulfills GMP requirements.

If you use equipment that is not part 11 compliant and you step into clinical phase, this equipment will not be accepted, so you must switch to new equipment. That means you may start from the beginning because you need to create new data, and the data might look different than what you have created so far.

You don't need to follow GMP rules in the documentation from early on, but the more you document, and the better you consider the aspects at an earlier stage, the better and more smoothly you will move forward from Phase 1 to Phase 2, Phase 3, and into commercial.

JH: Horst is absolutely right; data integrity is critical and many developers miss that. In addition, there are other aspects that academic, or early-stage gene therapy developers often don't consider. Therefore, I would suggest that a gene therapy developer contact experts like Charles River lab as early as possible. We're happy to provide consultative suggestions and services so they are staged for success very early on. It pains me to see programs that are not staged or designed well, so that we have to rework a lot of design, which wastes a lot of time and money.

Q What are the specific benefits to the integrated solution that the combination of Cobra Bio, Vigene Biosciences, and CRL provides to the advanced therapies community?

JH: I think first and foremost is speed. Charles River now has an integrated end-to-end solution from plasmid cell supply to viral vector and testing capability.

I would like to actually start from the end – testing. The testing takes as much time as

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- Jeffrey Hung

the manufacturing of cells, so if we can integrate the manufacturing with testing, and build in a lot of preparation work, that can save a lot of time and money for a gene therapy development program.

DS: From my perspective, we are able to offer customers choice, with multiple entry points to their development and manufacturing approach. Some developers will want the full service – plasmid, viral vectors, and cell therapy manufacture, all tested through the biologics function. Others will want to dip in and out at different points of that. Our integrated approach allows us to offer that choice to customers, and ultimately help them reach their patients quicker than they otherwise could have done.

HR: Testing and characterization are critical to the quality and safety of the product, and Charles River has been doing that testing for more than 20 years. Integrating this expertise and experience into the CDMO space ensures best testing strategies and strong support if you run into trouble with testing results.

If the client gets everything from one place, they don't have to manage multiple master service agreements and leaves them free to focus on what matters – getting therapies to patients.

BIOGRAPHIES

Jeffrey Hung, PhD

General Manager, Vigene Biosciences, a Charles River Company

Dr. Hung has over 20 years of experience in the gene therapy, synthetic biology and drug development. He joined Vigene in 2016 and orchestrated the acquisition of Omnia Biologics, a GMP manufacturer of viral vectors of 15 years. He has also overseen Vigene's expansion into GMP manufacturing and new product areas such as biosensors. An experienced entrepreneur, Dr. Hung was instrumental in successfully growing GenScript and SABiosciences, two previous companies, to IPO and acquisition stage, respectively. He also previously held the position of Chief Marketing Officer at ATCC. Jeffrey is the author of multiple patents, publications, and book chapters. He holds a PhD in genetics from Cornell University, an MBA from UC Berkeley, and a B.S. in biology from Peking University.

Professor Daniel C Smith, PhD, FRSB

Executive Director, Global Cell and Gene Therapy Portfolio, Cobra Biologics, a Charles River Company

Following the acquisition of Cobra Biologics/Cognate BioServices by Charles River Laboratories (CRL) in April 2021, Professor Smith was appointed Executive Director, Global Cell and Gene Therapy Portfolio within the CRL Corporate Development and Strategy function. Prior to acquisition, Professor Smith was the Chief Scientific Officer across the Cognate/Cobra CGT CDMO portfolio (2020–2021), and Cobra Biologics (2014–2020), driving CDMO innovations and partnerships across plasmid DNA, viral vectors, and latterly, (gene-modified) Cell Therapies, with respect to development and production. Previously he was Knowledge Transfer Manager and Senior Technologist for BioProcessUK. Daniel spent five years (2005–2010) at Cobra in roles including Senior Scientist, QC Team Leader, Head of Process Technology Transfer and Commercial Scientific Development Manager. He has over 8 years academic research experience, 30+ research publications to his name and a PhD in Molecular Cell Biology, and a BSc (Hons) in Biochemistry. Daniel holds Honorary Industry Professor positions at both the University of Kent, UK and at the University of Warwick, UK.

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Horst Ruppach, PhD

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Horst Ruppach studied chemistry at the University of Cologne and the University of Marburg, Germany, and earned his PhD in virology (HIV) at the Georg Speyer House, Frankfurt. He has 25 years of experience in the field of virology. His expertise is in virus safety testing and virus/prion clearances studies requested for all biopharmaceuticals and medical devices using animal- or human-derived materials. Dr. Ruppach is currently responsible for the business development of Charles River's viral clearance and virology service worldwide.

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