Cell & Gene Therapy Bioprocessing & Commercialization 2020

Post-event Report

Introduction & Contents

Welcome,

Cell, gene, and tissue therapies continue to be the fastest growing fields in the biopharmaceutical industry.

These advanced medicines are treating complex diseases and conditions such as cancer, tissue injuries, and revolutionary vaccines.

But significant processing and analytical challenges remain, however.

Every year, Informa Connect invites cell, gene, and tissue therapy industry representatives meet to discuss best practices and best strategies for overcoming those challenges.

Like most meetings in 2020, the **Cell and Gene Bioprocessing and Commercialization** conference was offered entirely online.

During 19–22 October, participants were invited to listen to presentations, plenaries, speaker panels, and even live virtual laboratory tours.

The five tracks were cell therapies, in vivo gene therapies, ex vivo gene-edited cell therapies, and advanced tools for cell and tissue manufacturing.

This report highlights a few presentations and speaker panels (by topic).

Maribel Rios,

Managing Editor at *BioProcess International*

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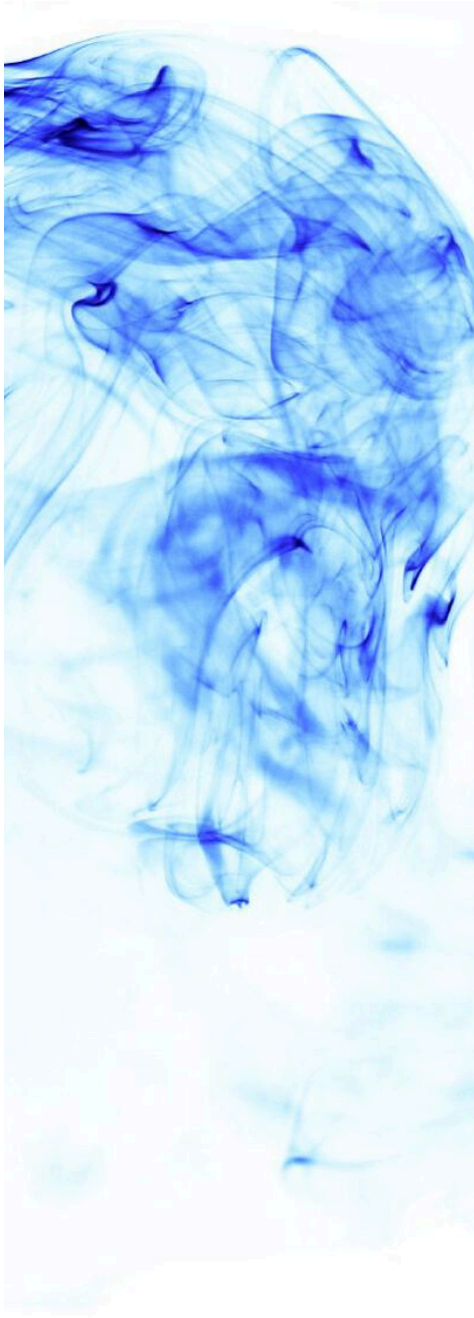
Panels & Plenaries

Investment, viral vectors, CAR-T and COVID-19

Panels and Plenaries

Some of the most exciting talks were found in the live agenda, as hundreds of attendees tuned in to join the panel discussions and plenaries. Here, **Maribel Rios**, Managing Editor at *BioProcess International*, reflects on the most popular live sessions across the week.



Investment in the CGT space: Trends, Technologies, and Early Stage Success

Led by Mike Zhao (MSQ Ventures), the panel included Miguel Forte (Bone Therapeutics), Jak Knowles (Leaps by Bayer), Joey Mason (M Ventures), and Dominic Schmidt (Syncona Investment Management Ltd.).

The discussion began with projections about the future of allogeneic and autologous cell therapies and whether "off-the-shelf" allogeneic products would eventually be the most preferred platform over autologous formats.

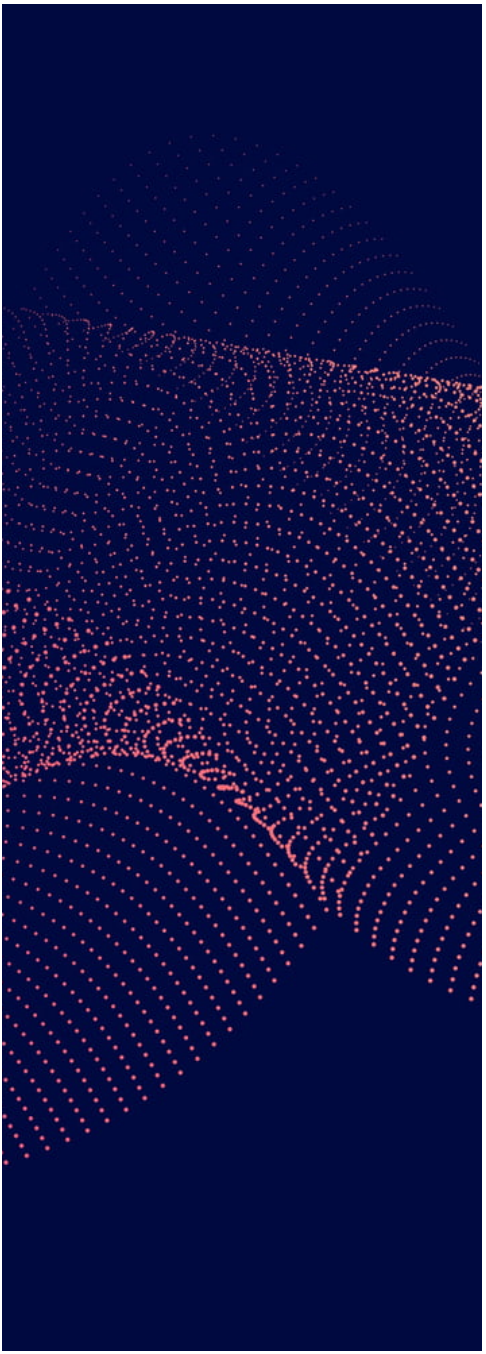
Schmidt and others agreed that both types would be a part of the industry in the long term. For example, autologous chimeric antigen receptor (CAR) T cell therapies are beneficial for certain indications.

"Ultimately what matters is efficacy and safety for patients," said Schmidt.

Forte also noted that the choice between autologous and allogeneic depends on the indication, the unmet medical need, cost, scalability, the value being brought by the final product, and other factors.

Mason added that manufacturing also is a critical factor. "There isn't a vein-to-vein process that can bring down the cost of these therapies, which are too expensive regardless of how they are created."

So new manufacturing technologies are needed that could reduce costs. The value of using contract



manufacturing organizations (CMOs) was another discussion point.

Knowles acknowledged that using CMOs would be helpful depending on the technology. “Most autologous therapies can be made using lentivirus platforms, for example, and there are many well established CMOs that can deliver GMP [good manufacturing practice] lentiviral transduction.

On the allogeneic side, people tend to prefer gene editing for allogeneic products, which is not achievable with lentivirus.

So companies that want to make allogeneic products tend to do the gene editing in-house, and the autologous programs tend to use CMOs to produce the lentivirus for transduction.

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> "Most allogeneic companies tend to focus on in-house gene editing, but autologous programs can use external production methods for lentivirus."
>
> Jak Knowles, VP Venture Investments, Head of Pharma Investments, Leaps by Bayer

From a pharma perspective, you prefer companies to build and own their technology so that you can get follow-on products.

But there is value in working with well-validated CMOs because most of them can scale-up for commercial launch.

The big difference that I’ve noticed is that most allogeneic companies tend to focus on in-house gene editing, but autologous programs can use external production methods for lentivirus.”

Zhao asked panelists what they look for what “early success” looks like to them. Knowles acknowledged that the definition of early success depends on therapeutic modality and the underlying technology.

For example, in one case he gained confidence when a technology “climbed the species ladder” and had success in different animal models during early development.

Forte said the most important element is when a therapy progresses to human clinical trials and the initial clinical data becomes available.

“If you’re in the early stages and you get the early successes, you need to hone the technology that you have because you are still in process development and building your product,” said

Knowles.

"Later, if you're able to scale up, you may be able to partner and outsource. That is late-stage success. For early stage success, you need to hone your technology and have your clinical data to support it." Mason provided a "macroview" of what is likely to be successful in a company.

Investors have seen a lot go wrong over their careers. So if you're trying to 'give comfort' to your investor base, you need to understand the journey and where the critical pieces are that are missing, or need to be fixed, or need to be addressed as time passes.

You need to know how much it's going to cost you to get to those critical milestones in the clinic.

All of that would be very important as we look at deals. Early understanding of what your competition is likely to be and what technologies are out there are critical."

PANEL: COVID-19: Trailblazers in the CGT Industry

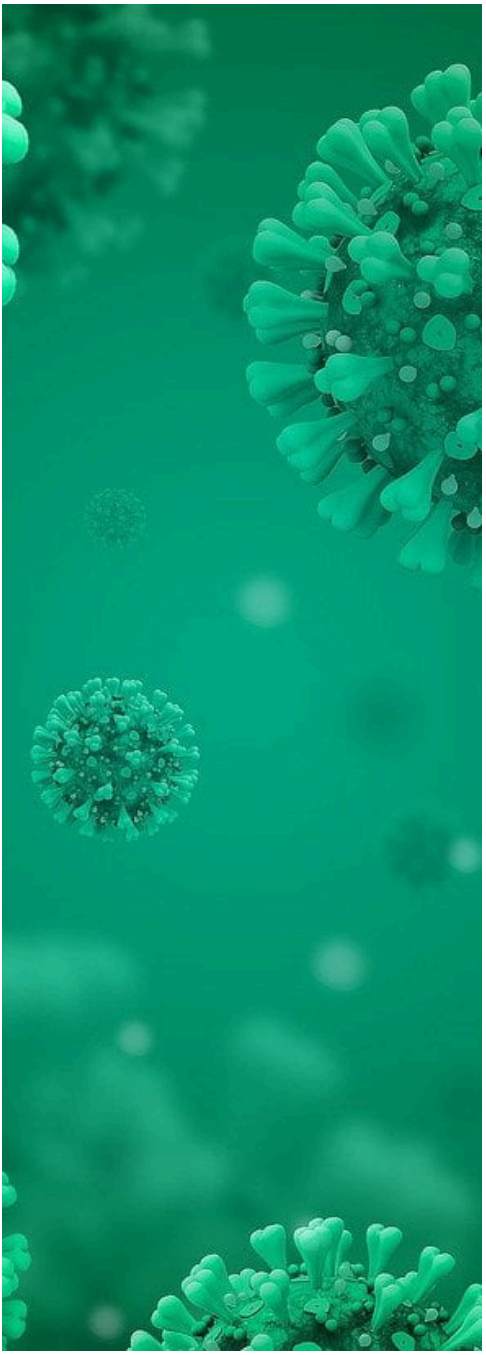
Led by Miguel Forte (Bone Therapeutics), the panel included Claudia Berron (Avantor), Chris Gemmiti (Sentien Biotechnologies), John Lewis (Aegis Life Inc. and Entos Pharmacetuticals), Racheli Ofir (Pluristem Therapeutics), Lawrence Thompson (Pfizer), and Camilo Ricordi (National Academy of Inventors, Italian Supreme Council of Health).

One of the main discussion points centered on the opportunities and challenges that biopharmaceutical scientists are facing regarding the COVID-19 pandemic.

All areas of the industry, including supply chain, have been facing uncertainties such as determining how to streamline R&D, production, and commercialization, optimizing processes, and staying in time.

These difficulties have been a part of all phases of cell and gene therapy development.

Ricordi noted that scalability was as one of the challenges, from hundreds of doses to thousands, specifically moving from two-dimensional expansion to



three dimensional bioreactors.

Other challenges were in the supply chain, including obtaining enough material to expand cells to the levels needed.

Panelists generally agreed that such “challenges” were results of the opportunities and the successes of a rapidly growing field and the needs to generate high volumes.

Other noted opportunities included the ability to obtain clinical readouts in short times, which allows researchers to obtain “good clinical proof” that therapies are working, and good cooperation with regulatory agencies.

Panelists also talked about the challenges brought on as a result of lockdowns (and other pandemic restrictions) and the opportunities to apply technologies used in response to COVID-19 for other indications.

Lewis pointed out that biopharmaceutical developers rely on a lot of partners, and many tasks must be completed in a short time.

Safety and product quality are not compromised but some risks are taken when multiple tasks must be done in parallel.

One challenge for developers has been to identify industry partners that are still used working in “typical” timeframe from discovery to clinic.

He also noted that this process also can be an opportunity because many potential partners (industry and regulatory) around the world are motivated toward the same goals.

Thompson chimed in on the discussion about the importance and challenge of having enough supplies (e.g., enzymes, lipids, and even syringes) to generate hundreds of millions of doses of a vaccine in a very short time.

“It’s amazing to do so much at the same time. When you are doing clinical trials but also doing late-stage manufacturing activities as if everything is going to work. Typically, we (Pfizer) doesn’t take these risks, but now we are doing everything at the same time.”

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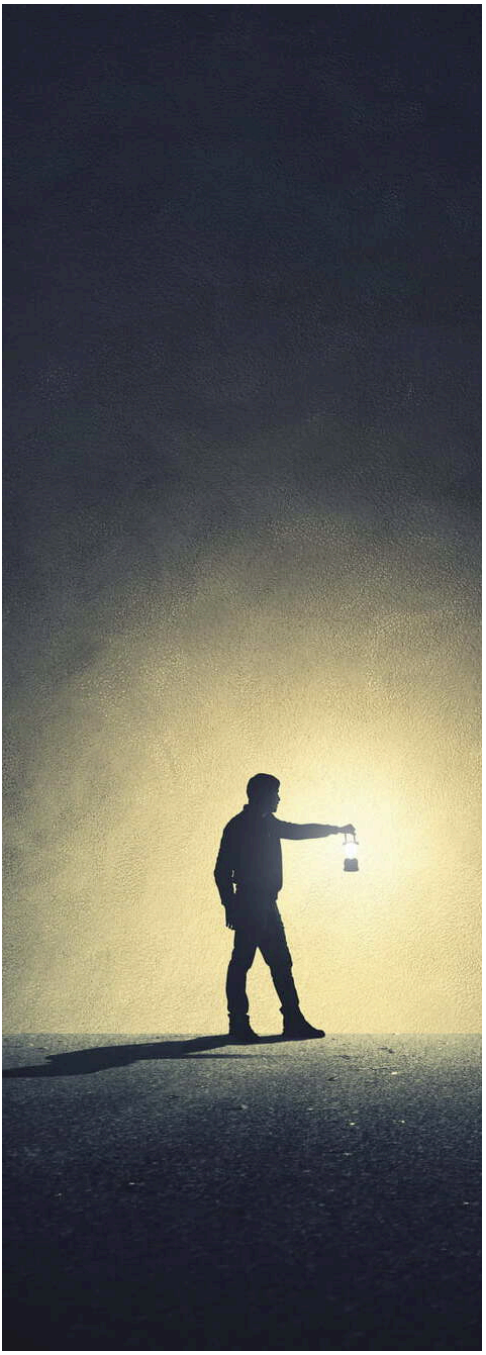
"Safety and product quality are not compromised but some risks are taken when multiple tasks must be done in parallel."



Raw Material, Viral Vector and Supply Chain Considerations

Led by Christopher Bravery (Advbiols), the panel included Scott Burger (Advanced Cell and Gene Therapy), Christiane Niederlaender (formerly MHRA, AMBR Consulting Ltd), Max Sellman (Aldevron), and Tom Walls (Spark Therapeutics).

The discussion began with everyone stating their biggest supply chain issue with either starting or raw materials.

One of the main difficulties that was noted is determining the difference between starting and raw materials from a regulatory perspective (such as for making viral vectors).

Panelists pointed out that the European Union has some documentation on the difference between both terms, but the US Food and Drug Administration does not.

Other supply chain challenges included minimizing variability in cellular starting material (especially for autologous cell therapy products), mitigating risk with single- and multiple-sourced suppliers, and maximizing control and consistency of an apheresis process.

Panelists pointed out that even before the pandemic, biotherapeutic developers faced problems with changing lead times and delays, especially for single-use assemblies and bulk media materials.

> "Suppliers must either buy reference materials or prepare them in-house when a reference product is not available"

Other discussion points focused on the shortage of certified reference materials and the challenge of handling variability of activity in those materials.

Suppliers must either buy reference materials or prepare them in-house when a reference product is not available (which, as one panelist pointed out, is the case for most items in a bill of materials).

And GMP facilities typically conduct biological activity assays on materials when received (if a standardized assay is available).

Many materials, however, cannot be tested against compendial standards, so those materials can be difficult to source.

How to ensure activity and consistency of a materials such as a cytokines are "open questions for which we don't have answers for," said Bravery. "But they are something that we need."

Data-Driven Strategies for Downstream Processing of Plasmid DNA In Viral Vector Production

Thomas Parker (MilliporeSigma) explained that plasmids are double-helix DNA molecules that are found naturally in bacteria and that replicate intracellularly.

Of the different topological forms, the supercoiled plasmid is recognized by FDA as the most therapeutically effective.

Plasmid DNA (pDNA) vectors are used to produce viral vectors and vaccines (e.g., mRNA-based such as the current COVID-19 vaccines).

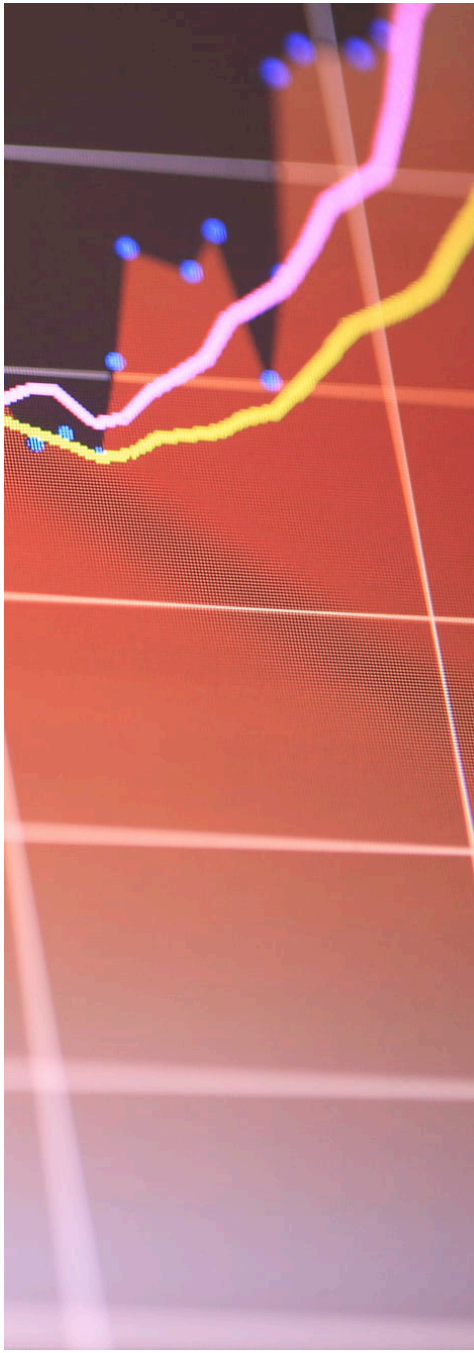
Parker presented an overview of all unit operations of plasmid DNA (pDNA) purification, from cell harvest to sterile filtration.

In gene therapy transient transfection, four plasmids (including the plasmid coating for the transgene) are used to make a viral vector encapsulating a gene of interest.

Parker presented a list of unique challenges of pDNA purification. They included low productivity of microbial fermentation, high viscosity fees, shear sensitivity, and low-resolution separation.

Parker showed a general plasmid process, highlighting where MilliporeSigma technologies and services can be implemented to provide an "integrated approach from harvest to final fill."

He also reviewed the approaches and key considerations for each pDNA processing step, providing case studies and operating parameters for some steps.



Non-Gene Edited Approaches to Allogeneic CAR-T Cell Therapy

Philippe Parone (Celyad) highlighted two platforms developed by Celyad: a T-cell receptor (TCR) inhibitory molecule (TIM) technology and a short hairpin RNA (shRNA)-based allogeneic platform.

The TIM platform is being used for the development of the company's allogeneic chimeric antigen receptor CAR T-cell candidate for metastatic colorectal cancer.

"TCR complex is responsible for graft versus host disease (GvHD) and attenuation of the TCR complex is necessary for creating CAR-T therapies," said Parone.

When TIM is overexpressed from a T cell, it significantly attenuates CD3-zeta. So, TIM-based allogeneic CAR T-cells do not exhibit in vitro and in vivo alloreactivity.

The company also has developed its NKG2D receptor, which is coexpressed by the company's CYAD-101 allogeneic candidate (for metastatic colorectal cancer) along with TIM and selection marker for an "all in one" vector approach.

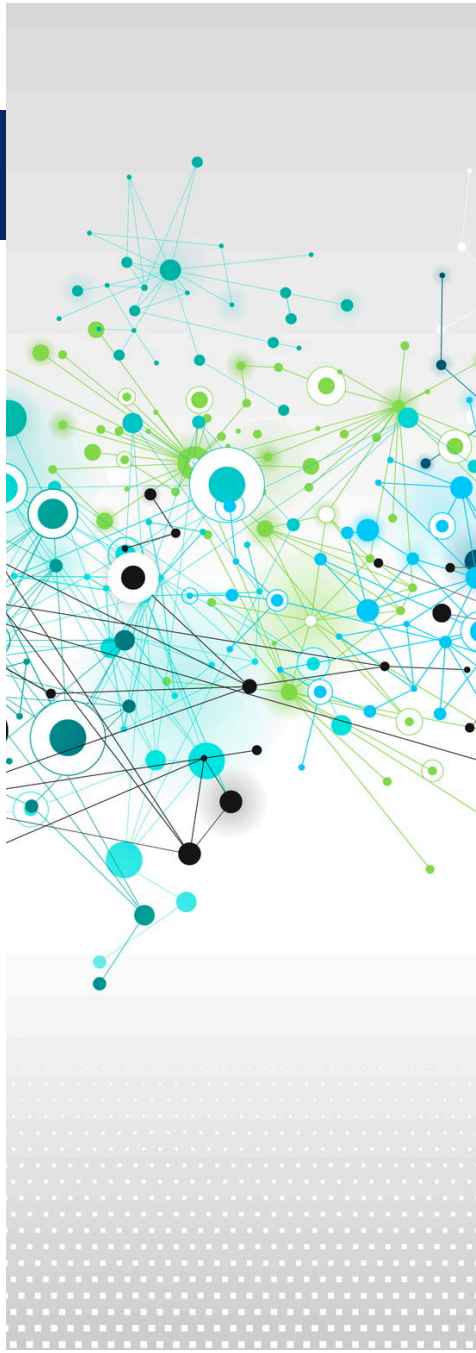
The company's shRNA platform is being used for the development of an allogeneic CAR-T candidate for multiple myeloma.

The company collaborated with Horizon Discovery Group, which provided its SMARTvector technology that mimics the endogenous nature of microRNA. shRNA enables knockdown gene expression through RNA interference.

Parone showed that shRNA targeting CD3-zeta reduces TCR expression with no overt transcriptome disturbance. Expression of a single shRNA also provides prolonged TCR knockdown without inducing GvHD.

The size taken up by the shRNA on the vector also is relatively minimal (250 bp).

Parone showed results of the platform's capability in multiple gene knockdown and concluded with an overview of the company's CYAD-200 series of shRNA-based allogeneic CAR-T candidates.



The Potential of CAR-NK

Kathryn Corzo (Takeda) presented the limitations of current CAR-T platforms.

They include limited access (half of patients require ICU management), failure to treat (manufacturing failures can delay or prohibit treatment), and complex supply chain (as a result of patient-specific manufacturing).

The company partnered with MD Anderson Cancer Center to develop CAR-NK (natural killer) cell therapies for patients with non-Hodgkin's lymphoma. Corzo highlighted the company's TAK-007 candidate based on that platform.

The allogeneic therapy is engineered with interleukin (IL)-15, which enables multiple ways of recognizing tumors. The first target is CD19. Corzo showed early clinical results and future development plans.

She concluded by highlighting the company's prioritization of data science and digital tools for rapid decision-making, including data governance, machine learning, and data visualization.

Starting Material Standardization

Christiane Niederlaender (formerly MHRA, AMBR Consulting Ltd.) focused on internal (within a manufacturing site) standards to ensure quality control and product consistency and highlighted the benefits of standardization of procedures and acceptance criteria, especially for starting materials.

She discussed the issues of grade designations for starting and raw materials, and how realistic it is to provide international standards to the ATMP (advanced therapy medicinal products) field.

In the European Union legislation, starting materials are defined as "all materials from which the active substance is manufactured

> "Characterization is less challenging for noncellular starting materials for gene therapy medicinal products. However, quality and purity of such materials are major concerns."

or extracted." In the United States, such materials are typically referred to as "critical raw materials."

EU regulations also define raw materials as "any other substances used for manufacturing or extracting the active substances, but from which this active substance is not directly derived (e.g., reagents culture media, buffers).

Niederlaender focused on regulations pertaining to genetically modified cells (EC Directive, Part IV Annex 1, paragraphs 3.2.1).

Although there are external standards for procurement and collection procedures of some cells (e.g., bone marrow, apheresis products, and blood-derived sources).

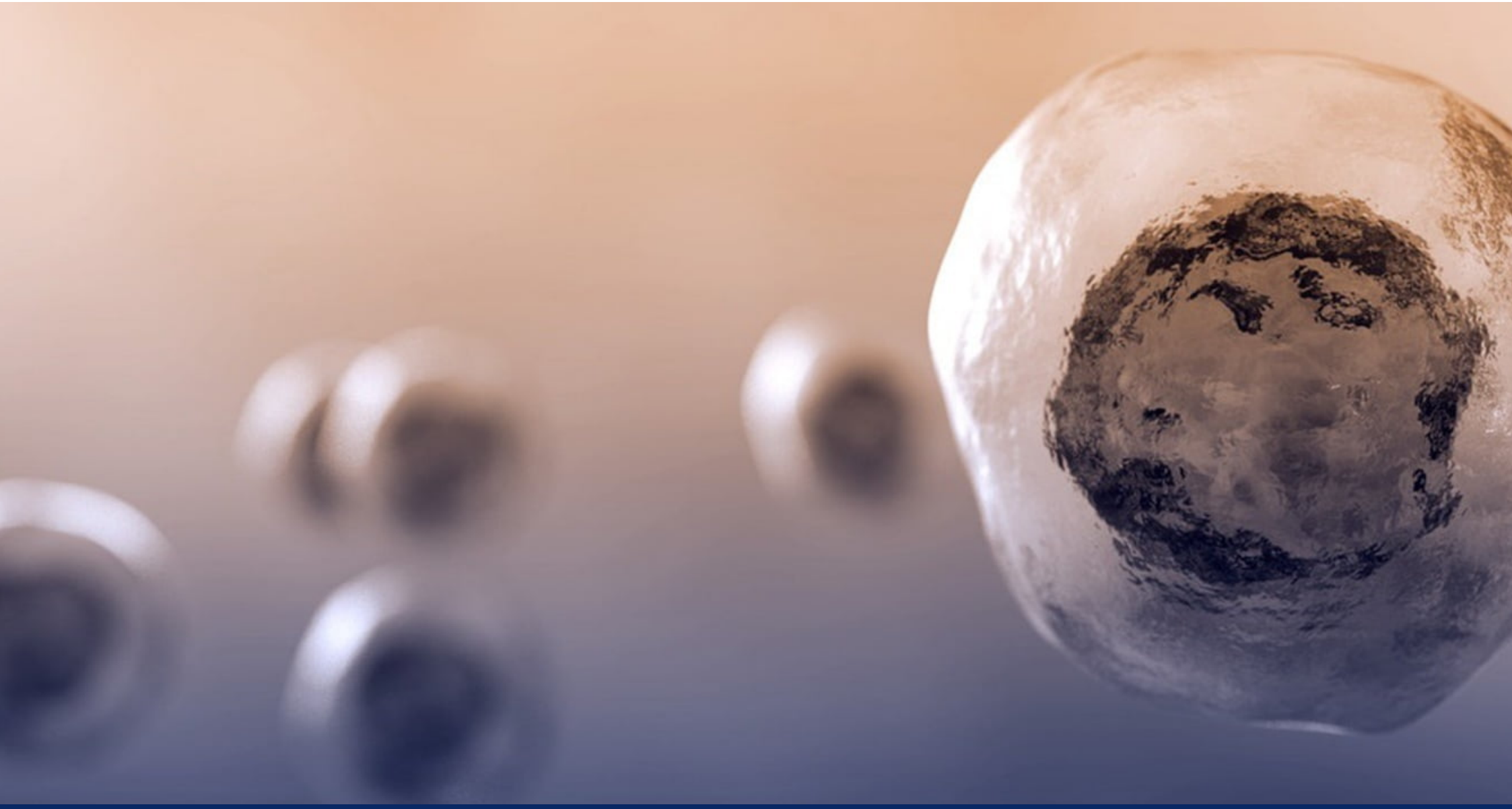
"However, even in these situations there needs to be some patient-specific and product-specific flexibilities."

Characterization is less challenging for noncellular starting materials for gene therapy medicinal products (e.g., viral vectors, plasmids, gene-editing components).

However, quality and purity of such materials are major concerns.

She explained how the use of "supplier grades" have no clear regulatory definition and proposed that manufacturers instead rely on in-house testing, change control, recognized quality systems, and qualify suppliers based on risk.

Niederlaender said that ideally, regulators would certify starting and raw materials, but she said there are difficulties with this concept, including how the required attributes would be defined and the increased resource constraints that would be put on regulatory agencies.



Cell Therapy

Automated Analytics and Autologous CAR-T Cell Therapy

Arguably the biggest conference track of the event, **Cell Therapy** provided live and on-demand sessions exploring topics from process development and manufacturing to analytical technologies.

Maribel Rios looks back at two of the most popular sessions across the week.

Cell Therapy

Augmenting Automated Analytics for Cell and Gene Therapy Using Fluorescent Nanosensors

Veeren Chauhan, (University of Nottingham) explained that traditional bioreactor sensors measure extracellular parameters such as pH, cellular oxygen, and metabolites to ensure that cells are growing effectively.

However, most biochemical changes that are important for cell growth occur on a subcellular level, including subcellular molecular oxygen, protons in the form of pH, carbon dioxide, and macromolecular structures such as cytokines and proteins.

New measurement technologies are needed to enable subcellular measurements, and automated analytics are needed to enhance efficacy of cell culture.

> "New measurement technologies are needed to enable subcellular measurements"

Such sensors ideally should be noninvasive and highly sensitive and provide high spatial and temporal resolutions.

Fluorescent nanosensors have been developed that can be delivered to subcellular spaces and make continual long-term measurements.

The compose of a biologically friendly (inert) matrix that protects

cells from dyes and fluorophores from cellular interferents.

They also contain an analyte and a reference fluorophore. The sensors permit silent report of key biological parameters, accurate ratiometric measurements, and high spatial and temporal resolutions.

Chuahan provided details of a study that used nanosensors sensitive to pH (acid test.

The sensors contained a polyacrylamide matrix (50-nm in diameter) and composed to two pH-sensitive fluorphores (Oregon Green and carboxyfluorescein), and pH-sensitive rhodamine.

With all three fluorophores, the sensors could make pH sensitive measurements pH 3.5 to pH 7.5.

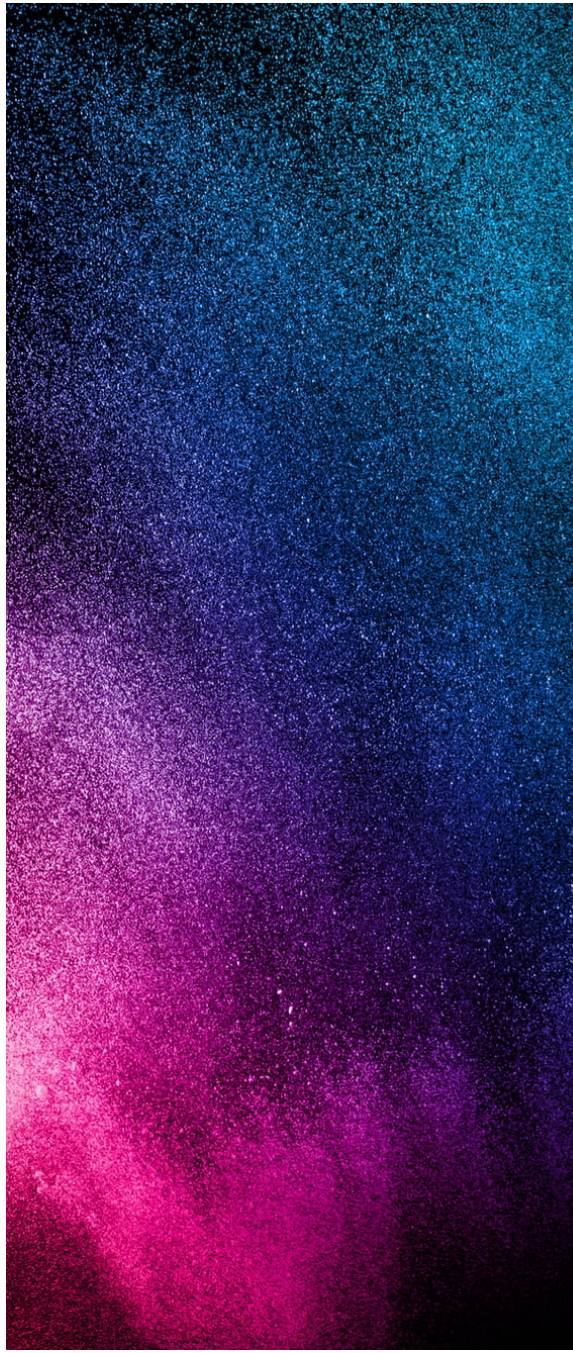
A calibration curve could be generated by taking a ratio of the intensities of Oregon Green and rhodamine at specific pH.

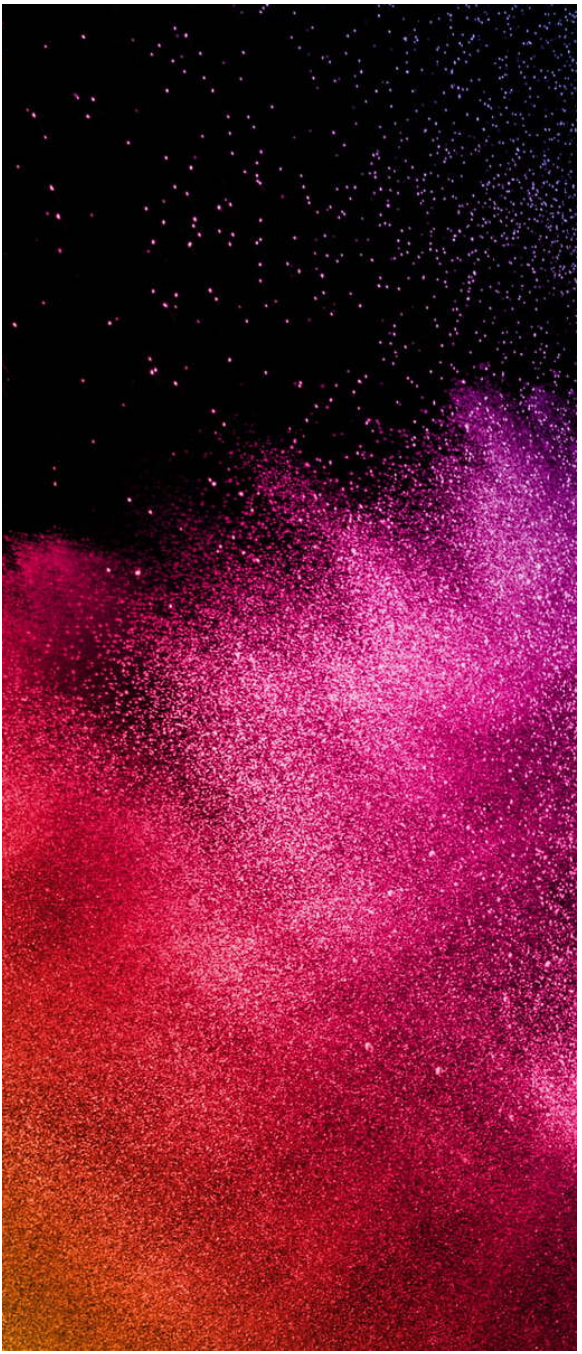
Chuahan suggested that the accuracy of the nanosensors (+/- 0.17 pH units) makes them ideal for online measurements in biological systems.

They also can provide real-time subcellular analytics and contribute to the optimization of each stage of cell and gene therapy manufacturing.

Chuahan also provided examples of how fluorescent nanosensors can be used to make complex measurements in C. elegan and eukaryotic cell lines.

They also can be used to augment automated analytics in cell and gene manufacturing processes (off-line, on-line, and in-line) by monitoring biochemical subcellular changes.





Risk-Based Approaches for Autologous CAR T-Cell Therapy Comparability

Erica Brust (Bristol Myers-Squibb) emphasized that the goal of comparability as defined in the ICH Q5E guidance is "to ensure quality, safety, and efficacy of drug products . . . produced through collection and evaluation of relevant data."

After an overview of ICH Q5E and EMA guidelines, she focused on phase-dependent approaches to comparability, taking into the account the lifecycle of drug products.

For example, first-in-human studies typically do not require comparability studies, but during phase 2 and 3, a risk-based approach should be taken to determine the scope and tiers for comparability assessment.

For paired run studies, risk-developers typically "split either at the source material or further downstream, depending on the nature of the change."

Burst presented a general risk-based comparability strategy flowchart based on assessment of process control and critical quality attribute assessment and a general approach to beginning a comparability assessment.

One approach to comparability risk assessment is scoring, in which attributes are assigned a number based on potential risk of a change to cause variation in process performance.

Those scores are used to prioritize which aspects to assess in the comparability assessment.

A three-tier approach to comparability risk assessment also has been a successful strategy.

Brust provided and overview of the tiers (generally: tier 1 is equivalence test approach, tier 2 is quality range, tier 3 is visual assessment approach), and concluded with a case study of risk-based comparability assessment for drug product manufacturing site transfer.

In Vivo Gene Therapy

Next Generation Cell-line Development and Recombinant Adenoassociated Virus Production

From an informative selection of insights across upstream and downstream bioprocessing and vector manufacturing, we review the most watched presentation in the **In Vivo Gene Therapy** track.

In Vivo Gene Therapy

Next Generation Cell Line Development and Large-Scale Bioprocessing Technology

Sam Wadsworth (Ultragenx Gene Therapy) began by highlighting the features of his company's gene therapy manufacturing processes based on human embryonic kidney (HEK)293 suspension/plasmid transfection and HeLa producer cell line (PCL) suspension/adenovirus helper platforms.

In a collaboration with Bayer, the company has conducted engineering improvements to its HeLa PCL platform.

Specifically, the company made molecular changes to the plasmid components and multiple process and screening changes to the original "HeLa 1.0" platform.

The improved "HeLa 2.0" stage has a 10× increase in yield and a streamlined clonal selection.

The technology now is used for the company's work in finding a gene therapy for Wilson's disease.

Wadsworth showed how further improvements to the platform ("HeLa 3.0") could be used to increase production of adenoassociated virus (AAV) vector yield and how the use of a siRNA knockdown of specific genes could improve AAV production titer.

He highlighted work on Wilson's disease potency assays and the use of clustered regularly interspaced short palindromic repeats (CRISPR) CRISPR-associated protein 9 (Cas 9) to delete ATP7B (the loss of this function causes copper toxicity in Wilson's disease) in the HepG2 cell line.

Gene-Edited Ex Vivo Cell Therapy

Gene Modified Allogeneic MSCs and NK Cells

Gene-Edited Ex Vivo Cell Therapy

Senti Bio Case Study: Gene Modified Allogeneic MSCs and NK Cells

Philip Lee (Senti Biosciences) introduced his company's "gene circuit" platform, which is a genetic "logic system" created by a method of combining DNA.

Lee also discussed a case study focused on the use engineered mesenchymal stem cells (MSCs) to treat solid tumors through lentiviral vector transduction.

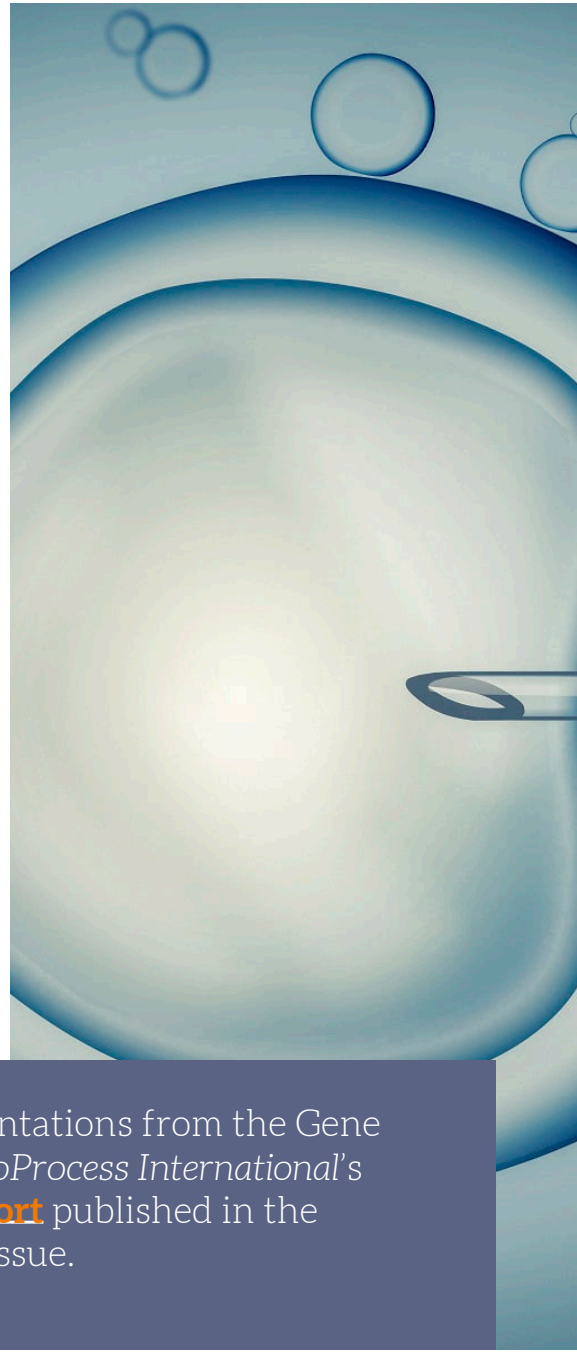
The cells were derived from bone marrow and engineered to express two immune-stimulated payloads (interleukins 12 and 21).

Data from preclinical trials that showed that the combination of those payloads is highly potent and that it yields a robust antitumor response across every tumor model evaluated.

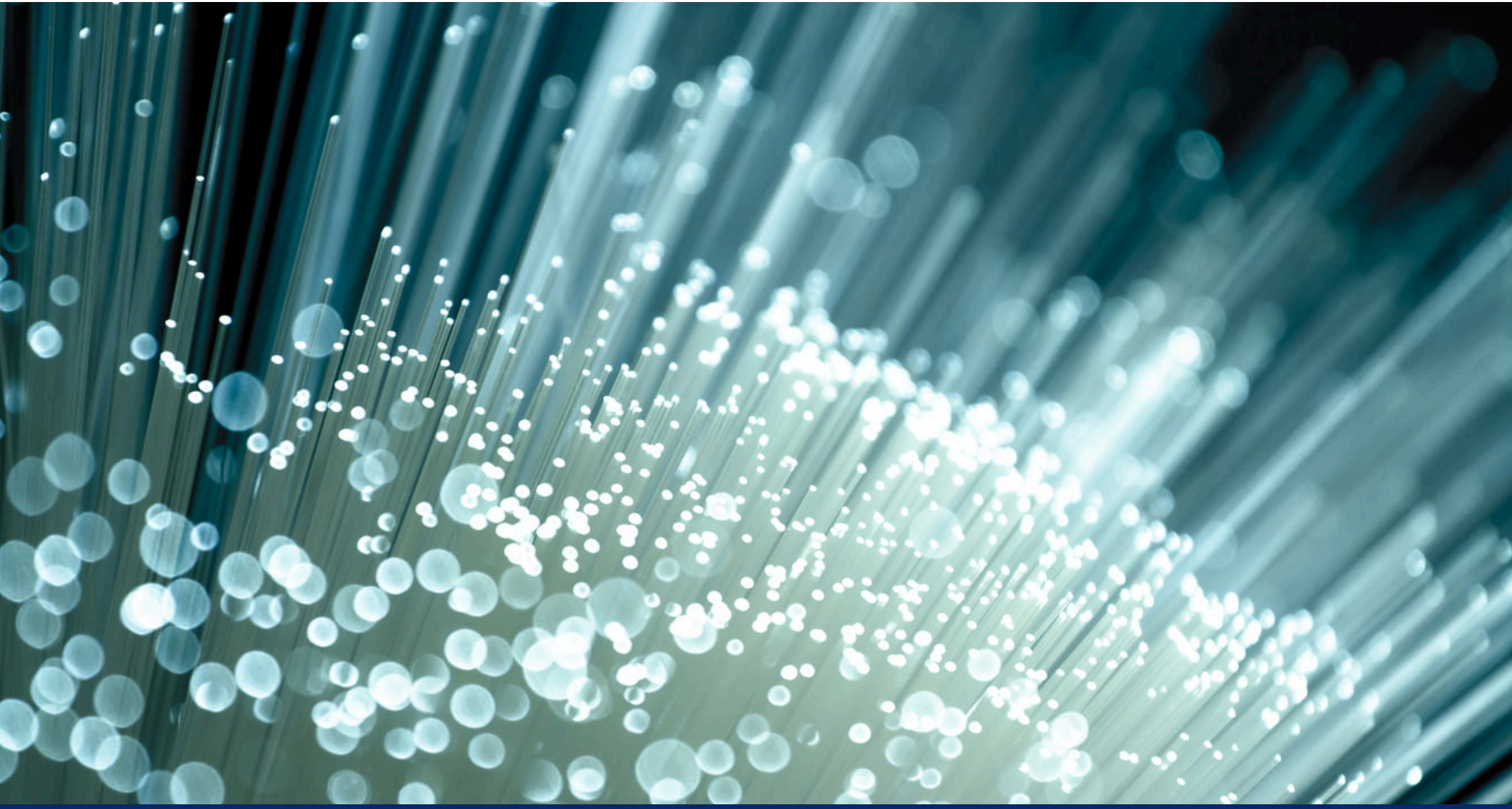
Lee presented his company's strategy for transferring its genetic modification process to good manufacturing practice (GMP) scales.

He also showed how the MSC platform can be expanded to other gene-modified cell therapies. The second study focused on the early stage development an allogeneic natural killer (NK) cell treatment for cancer.

Lee provided an overview of the Senti Biosciences's method for robust cell isolation and cryopreservation and emphasized the company's continued work in optimizing its viral vector process and NK cell transduction and expansion strategies.



For overviews of more presentations from the Gene Therapy tracks, check out *BioProcess International*'s **Gene Therapy Featured Report** published in the November/December 2020 issue.



Advanced Tools for Cell and Tissue Manufacturing

Diverse Tissue Engineering Applications and Automated Cell Harvesting

Advanced Tools for Cell and Tissue Manufacturing

Development of Modular Biofabrication Platforms for Diverse Tissue Engineering Applications

George Joseph Christ (University of Virginia) began with an overview of the importance of treating volumetric muscle loss injuries.

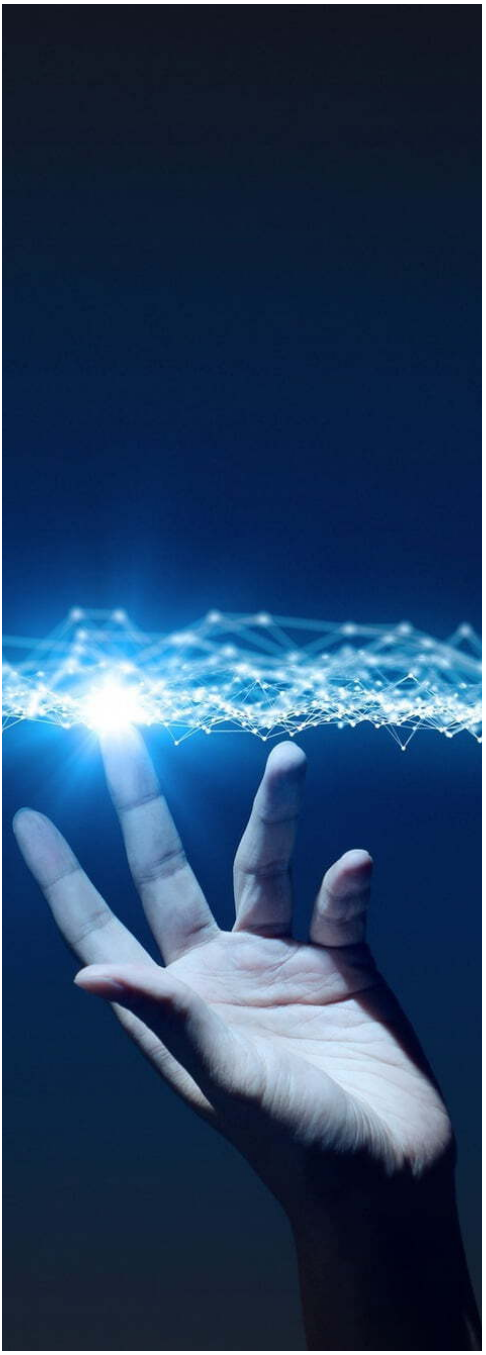
He presented data supporting the application of tissue-engineered muscle repair (TEMR) constructs in animal models and tissue engineering to repair volumetric muscle loss injuries (face and hand) and the use of sheet-based tissue-engineered medical products (TEMPs) for muscle repair.

He presented data showing the benefits of adding seeding cellular material on an extracellular matrix scaffold (2-cm square area), comparing TEMR (with cells) results (e.g., volume quality, composition, vascularization, contractile force) with those of applying bladder acellular matrix (BAM) alone.

Christ reviewed his group's TEMR implant process and manual engineering process, including taking a biopsy from a patient, expanding progenitor cells, matrix seeding (manually), tissue stretching, and preconditioning of skeletal muscle constructs in a bioreactor for in vitro maturation.

"The idea is that we are not implanting mature muscle. We are implanting myoblasts and myotubes (mainly myoblasts) to create a regenerative template, which enhances the microenvironment for tissue repair that normally would not exist in adult mammals to improve tissue repair."

Christ compared the traditional manual TEMR creation process with automated bioprinting.



"We envision an automated bioprinting method whereby we can take the same construct, put it in a holder in the same bioreactor used for the TEMR approach, and bioprinting instead of manually seeding at a higher initial cell density with fewer cells in a rapid process, and then placing the system back into the bioreactor."

He summarized the TEMR approach as being a "hybrid" biofabrication process between bioprinting and cell sheets and showed a process (and challenges) for bioprinting tissue-engineered medicinal products (TEMPs).

Automated Cell Harvesting Isolation Technologies

Sonia Bulsara (Cytiva) presented the company's technology for each stage of a cell therapy workflow.

She focused on capabilities and applications of the Sepax C and Sefia cell processing and isolation instruments.

The former is composed of the hardware, protocol software, and cell processing kit.

The automated system is closed for compliance to good manufacturing practice (GMP) applications. The Sepax instrument uses centrifugation for cell separation, as specified by a protocol.

The optical detection to sense the transition of layers between the cells that have been separated so that the cells can be pulled to the appropriate bag.

Applications include harvesting of mesenchymal stems cells (MSCs) and T cells.

The Sefia instrument was developed to address scale up to handle larger volumes (up to 10 L) and higher capacities.

It includes sensors for weight, temperature and enables eight fluid pathways. The instrument can be implemented in continuous flow processes.

Applications for the Sefia system include isolation in upstream manufacturing workflow (PremierCell protocol) and automated cell harvest (FlexCell protocol).

See you at

Cell Therapy Digital Week in February 2021