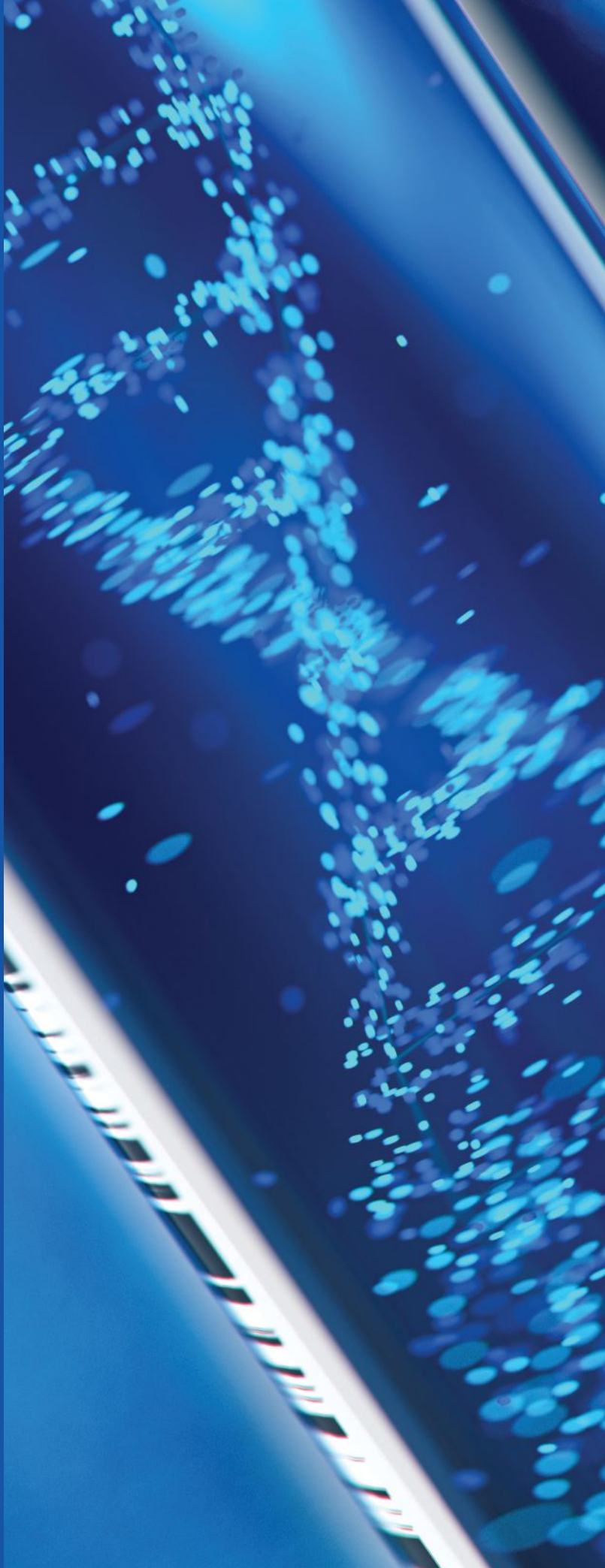
GEN Genetic Engineering & Biotechnology News

SUPPLEMENT

Cell and Gene Therapy

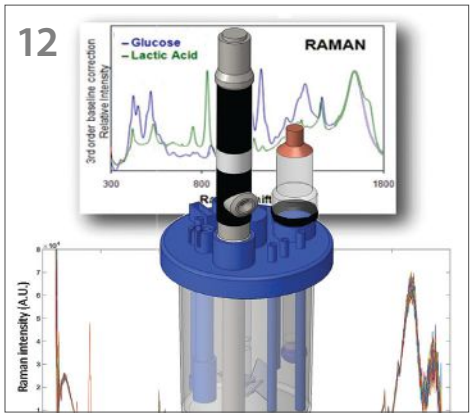


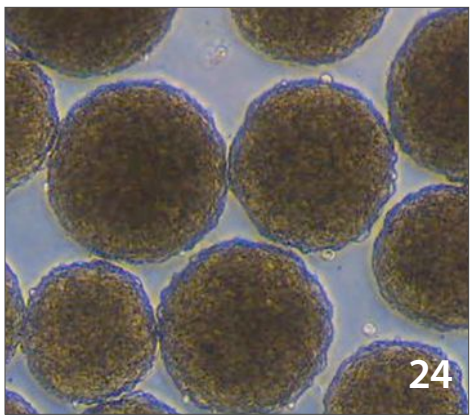
GEN Genetic Engineering & Biotechnology News

SUPPLEMENT

Cell and Gene Therapy

CONTENTS





Cover photo: Embyonic Stem Cells by Maksim Tkachenko/Getty Images

Cell and Gene Therapy
Never More Lively

Technological and manufacturing advances and streamlined regulatory processes contribute to a booming market

John Sterling
Editor in Chief

"The market for cell and gene therapy is growing at an exceptional rate because it represents a way to counter the growing incidence of chronic diseases. As the total addressable market is huge and offers tremendous growth opportunities, many vendors are making it a priority to develop and launch innovative and breakthrough cell and gene therapy products. Global governing bodies are offering regulatory support and granting fast-track, breakthrough, and regenerative medicine advanced therapy (RMAT) designations to expedite product approvals."

So writes Barath Palada, an analyst at Arizton Advisory and Intelligence and author of the lead article in this *GEN* supplement, which covers several key trends in cell and gene therapy. Appropriately, Palada introduces these trends, pointing out that many cell and gene therapy products, including chimeric antigen receptor (CAR) T-cell therapies, genetic modulation–based gene therapies, and autologous and allogenic cell-based therapies, are appearing for the treatment of chronic, rare, and genetic diseases.

Other articles in our supplement reinforce the point that robust and promising cell and gene therapy approaches are emerging. These articles also emphasize that the new approaches are being developed though varied technological advances. For example, advances in scaleup, process analytics, and regulatory affairs are discussed by Josh Roberts, who brings us his observations from Cell Therapy Manufacturing Asia 2020, a conference that was held in Kyoto, Japan, in February (p. 12).

Also reporting on a recent conference—Cambridge Healthtech Institute's annual PepTalk conference, which took place in San Diego in January—Catherine Shaffer emphasizes the event's many presentations on and cell and gene therapy. As her article demonstrates, Shaffer spoke to several experts who are working on novel techniques to improve the manufacture of cell-based therapies (p. 24). The same event informs the article by Mike May, who takes a different tack. Specifically, he describes the views of five presenters on viral vector–based gene therapies (p. 18).

Kevin Davies, PhD, *GEN's* editor at large, interviewed Guangping Gao, PhD, professor and director of the Horae Gene Therapy Center at the University of Massachusetts Medical School in Worcester. Gao shares his remarkable life journey and his hopes for the future of gene therapy (p. 33). We hope you enjoy this broad look at cell and gene therapies.

John Sterling
jsterling@GENengnews.com

AVTR
LISTED
NYSE

QUALITY

MOVES NEW THERAPIES *FORWARD*

Whether it's a vaccine, recombinant protein, or gene therapy, Avantor® can help transform how these therapies are brought to market, combining our unique workflow expertise with advanced biologic products engineered to satisfy the highest quality standards and regulatory requirements.

We help enable breakthroughs in life-changing biologics.

avantorsciences.com/moves-biopharma-forward

avantor™

Bright Outlook for the Global Cell and Gene Therapy Market

Factors accelerating market growth include successful product launches, a favorable regulatory environment, and sizable investments in research and development

By Barath Palada

Cell and gene therapies are highly advanced biological products that can treat chronic diseases such as chronic wounds, diabetic foot ulcers, pressure ulcers, and venous ulcers. They also help to cure bone-related defects, such as cartilage defects of the knee and ankle, and several cancers, including those affecting the blood, skin, prostate, liver, and kidney. These products have helped resolve rare genetic diseases, ocular disorders, and cardiovascular diseases (CVDs). Many cell and gene therapy products, including chimeric antigen receptor (CAR) T-cell therapies, genetic modulation–based gene therapies, and autologous and allogenic cell-based therapies, are available for treating chronic, rare, and genetic diseases.

The market for cell and gene therapy is growing at an exceptional rate because it represents a way to counter the growing incidence of chronic diseases. As the total addressable market is huge and offers tremendous growth opportunities, many vendors are making it a priority to develop and launch innovative and breakthrough cell and gene therapy products. Global governing bodies are offering regulatory support and granting fast-track, breakthrough, and regenerative medicine advanced therapy (RMAT) designations to expedite product approvals.

Market segmentation

Geography: The global cell and gene therapy market is segmented into five major regions: North America, Europe, Asia/Pacific (APAC), Latin America, and Middle East/Africa (MEA). In 2018, North America led the market with a share of 60%, followed by Europe, APAC, Latin America, and MEA.

Revenue: The United States is the largest contributor to both regional and global markets. In fact, North America has more than 400 companies that are actively engaged in research and product development of cell and gene therapy products. Europe is expected to be another prominent market due to the launch of new cell and gene therapy products. APAC is likely to emerge as a major market due to the high presence of chronic diseases in countries such as China and Japan.

Product type: The global cell and gene therapy market is segmented into gene therapy and cell therapy. Cell therapy was the dominant segment in 2018 and accounted for a share of around 76% of the global market. However, gene therapy products are likely to replace/outpace many cell therapy products by 2024 and to account for a market share of more than 50%, which is more than two times that of 2018. The launch of innovative and breakthrough products and the high adoption of CAR T-cell based therapies are the factors responsible for the growth of the cell therapy segment.

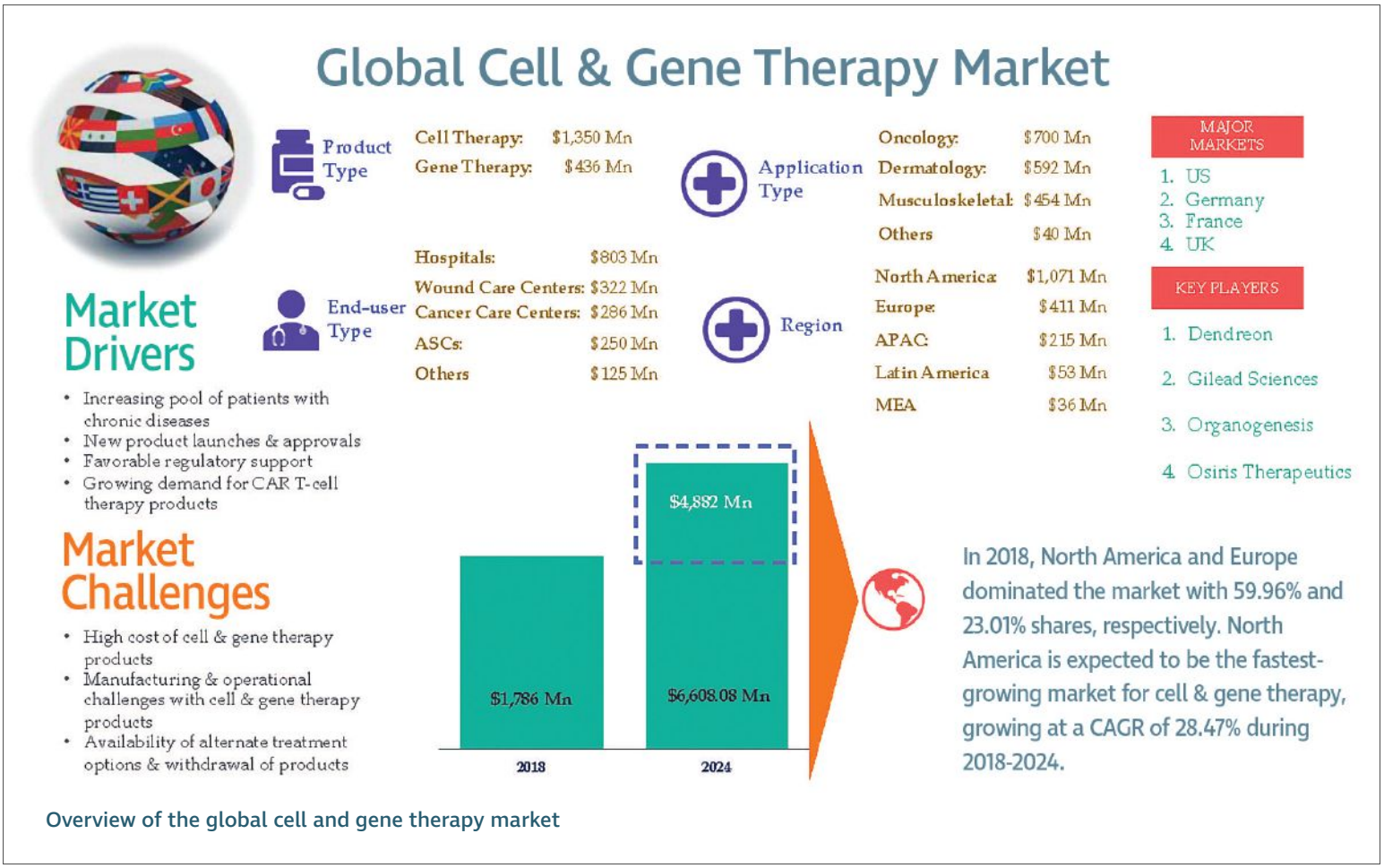
Factors influencing the market

Favorable patient demographics: The increased prevalence of several chronic diseases is contributing to growth of the cell and gene therapy market. Approximately one in three adults suffers from more than one chronic condition or multiple chronic conditions globally. Chronic diseases mainly include CVDs, cancer, chronic lung diseases, and diabetes.

Cell and gene therapy–based products have the capability to act as an effective and disease-modifying treatment, which could otherwise have been incurable through conventional therapies. The global cell and gene therapy market is witnessing double-digit growth due to the high demand for cell-based immunotherapies and gene therapy products to treat certain cancers such as prostate and hematological cancers.

The increasing use of cell therapy products for treating burns, venous leg ulcers, diabetic foot ulcers, injuries, and pressure ulcers in hospitalized patients is another factor contributing to market growth. According to the American College of Surgeons, approximately 1–2% of the global population experiences chronic wounds in their lifetime.

New product approvals/launches: Vendors are strategically focusing on the development and launch of single-use bioprocessing products to remain competitive and gain traction in the market. New product approvals and launches,



Overview of the global cell and gene therapy market

coupled with R&D activities, help vendors expand their presence, enhance market growth, and sustain market position in the global cell and gene therapy market. Vendors are also actively launching innovative devices to penetrate and tap the huge growth potential of the market. Novartis AG, Gilead Sciences, Vericel, MolMed, Anterogen, Organogenesis, Amgen, Dendreon, Orchard Therapeutics, and Spark Therapeutics have significant market shares worldwide due to their continuous involvement in product innovations and launches.

Strategic acquisitions/investments: At present, the cell and gene therapy market is witnessing significant M&A activity. Vendors, especially global players, are increasingly focusing on pursuing inorganic growth strategies such as acquisitions and investments to expand presence, enhance product portfolio, and improve expertise in the market. Multinational players such as Novartis, F. Hoffmann-La Roche, Gilead Sciences, Bristol-Myers Squibb, Celgene, and Pfizer are targeting emerging market players that have strong product pipelines.

Favorable regulatory developments: Several cell and gene therapy products are receiving regulatory approvals faster than other products due to superior safety and efficacy profiles for treating a broad range of diseases. Companies are launching these products in the market more quickly as the approval process is becoming more streamlined. The regulatory approval process, particularly in the United States, is evolving and becoming favorable for vendors for developing cell and gene therapy products. The U.S. Food and Drug Administration is designating orphan drug status, breakthrough designation, accelerated approvals, and RMAT designations for cell and gene therapies to expediate the approval process.

Therefore, the favorable regulatory support in the form of priority review, RMAT designations, orphan drug designations, and breakthrough designations will positively influence the demand from end users and provide major impetus to the market.

Growing demand for CAR T-cell therapy products: CAR T-cell therapies have gained significant traction in recent years. They represent the single most rapidly growing product type in the market, and they are generating revenue at a phenomenal rate. At present, CAR T-cell therapy is the fastest advancing technology in the treatment of cancer and has the capability to replace several existing therapies. CAR T-cell therapy addresses current challenges in cancer care through superior efficacy, safety, and

Bright Outlook for the Global Cell and Gene Therapy Market

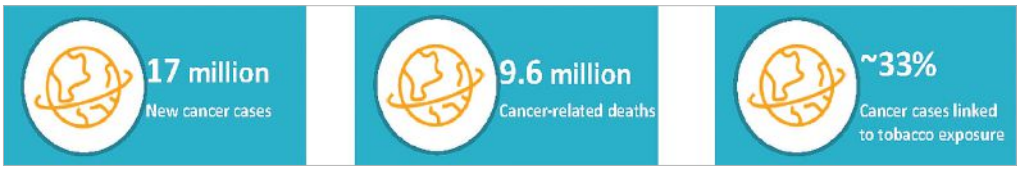
delivery mechanisms. CAR T-cell therapy has brought itself into focus due to highly personalized nature of this therapy and utilization of advanced genetic engineering technology.

CAR T-cell therapy has a few advantages that may not be available with other cancer-curing drugs. The most important advantage is remission that patients achieve in case of blood cancer. Once T cells are administered, they not only kill the tumor cells but also keep on growing and dividing. They provide long-lasting immunity against further tumor cell occurrence.

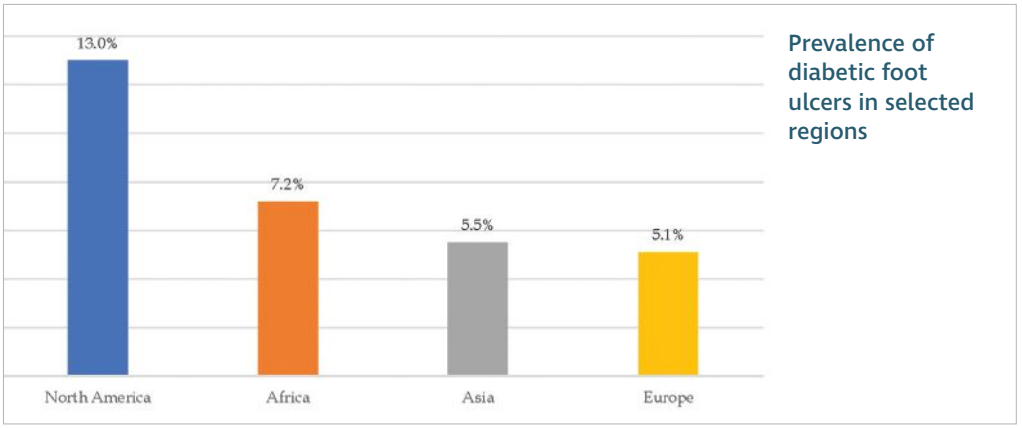
The landmark approvals of Yescarta and Kymriah have spurred extraordinary development in this field. The possibility of bringing a groundbreaking therapy to the market has sparked a wave of investment and innovation from pharma/biotech companies worldwide. Kymriah by Novartis contains tisagenlecleucel, and Gilead Sciences subsidiary Kite Pharma introduced Yescarta (axicabtagene ciloleucel). Kymriah is approved for both acute lymphocytic leukemia and diffuse large B-cell lymphoma, whereas Yescarta is approved for acute lymphoblastic leukemia and is currently undergoing a clinical trial for approval in diffusing large B-cell lymphoma. The potential for Kymriah and Yescarta to cure adults with aggressive B-cell lymphoma and selected pediatric and young adult patients with acute lymphoblastic leukemia is bringing new hope to those who previously tried other therapies.

The possibility of bringing a groundbreaking therapy to the market has sparked a wave of investment and innovation.

Owing to the huge market potential of CAR-T cell therapy, many companies have entered the field. In 2018, more than 90 CAR T-cell therapies were under investigation in more than 100 clinical trials worldwide. Small pharma/biotech companies are developing new CAR T-cell therapies, offering opportunities for acquisitions and collaborations. For instance, CARsgen Therapeutics alone has over 11 CAR T-cell therapy products, out of which 5 are in clinical trials.



Key cancer statistics (2018)



Prevalence of diabetic foot ulcers in selected regions

Increasing R&D funding: Many government organizations and private firms have started funding biotech start-ups and research institutes that are active in the R&D of cell and gene therapy products. Both public and private sectors are supporting cell and gene therapy developers. North America and Europe are at the forefront in terms of R&D activities and funding to develop and commercialize cell and gene therapy products.

For instance, Alliance for Cancer Gene Therapy awards both Young Investigator and Clinical Translation grants of $250,000 to $500,000 over a period of two to three years, inclusive of a maximum of 10% indirect costs. In May 2019, the California Institute for Regenerative Medicine and the National Heart, Lung, and Blood Institute entered a collaboration to co-fund and accelerate the development of cell and gene therapies to cure sickle-cell disease. This agreement was created under the National Institutes of Health's Cure Sickle Cell initiative.

The Medical Research Council in the United Kingdom is also funding cell and gene therapy. For example, it is funding research projects to improve the understanding of fundamental stem cell biology and regenerative processes, as well as development projects that apply regenerative technologies to improve human health. GEN

Barath Palada is an analyst at Arizton Advisory and Intelligence. Website: www.arizton.com.

Lonza

Pharma & Biotech

the next cell and gene therapy process...

Let's develop and industrialize it together.

True partnership
Our team aspires to become an extension of your team. We'll work side by side with you relentlessly to achieve your next milestone, while keeping your end-goal in mind.

A step-by-step approach
We'll follow a continuous improvement approach, while keeping your manufacturing costs under control, as your therapy progresses through the journey to commercialization and your priorities evolve.

Unmatched experience
Our highly experienced teams of experts are well-equipped to identify and address your production challenges early and de-risk your process, so you're not caught out when you need to scale up or out, and meet accelerated timelines for delivery.

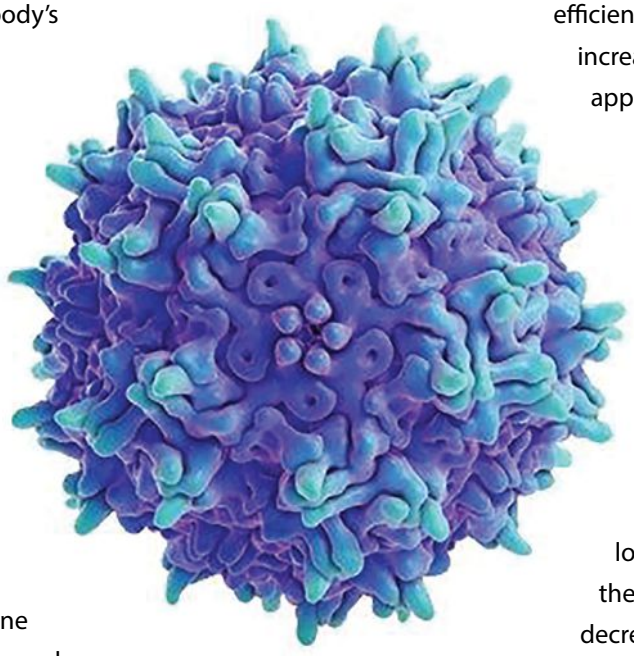
Dedicated regulatory support
Our dedicated cell and gene therapy regulatory consultants have decades of experience working with regulatory bodies and can help you to navigate the ever-changing regulatory landscape.

Contact us:
Visit pharma.lonza.com
Email pharma@lonza.com

Transformative Technology That Overcomes the Challenge to **Effective Gene Therapy**

Recombinant adeno-associated viral (AAV) vectors are among the most popular gene delivery systems for gene therapy. While AAV vectors were initially thought to be minimally immunogenic, innate and adaptive immune responses have been observed and implicated in the loss of transgene expression in human gene therapy clinical trials.

NxGEN Vector Solutions has a technology that can reduce or eliminate the body's immune reaction toward your AAV vectors, leading to safe, effective, and durable therapeutic gene expression. Our technology builds gene therapy vectors that are stealthy, coming in under the immune radar, permitting AAV vector delivery without activating the body's immune defenses. The breakthrough came by identifying the precise reason that the AAV vectors were setting off the body's alarm bells: unmethylated CpG dinucleotide motifs in the AAV vector expression cassette. By removing the CpG dinucleotide motifs, AAV vectors could be designed to deliver the curative genes without activating the body's immune system.



Clinical Evidence

A recent publication in *Molecular Therapy* detailed the data from eight gene therapy clinical trials, including variables such as the AAV serotype employed, the CpG dinucleotide content in the open reading frame of the therapeutic gene, the vector production method, the vector dosage and estimated total capsid dose, the immunosuppressive drugs administered, the correlation of AAV gene transfer with a cytotoxic T lymphocyte response (CTL) response, the outcome peak of the therapeutic transgene, and the durability of the transgene. The power of this clinical trial information is that it is the single largest AAV vector clinical data set for a single disease—AAV gene therapy for hemophilia B—and allows researchers to gain a clear insight into the key determinant for clinical success.

Among the variables, ***CpG content was revealed to be the only factor that correlates well with clinical outcome and long-term transgene expression, with unmethylated CpG content in AAV vectors being the key attribute that triggers transgene expression-limiting immune responses in humans***.

Codon modification to reduce the wild-type Factor IX (FIX) CpG content in the cDNA of the AAV vector expression cassette from 19 to 0 CpG motifs resulted in sustained FIX expression with the absence of or minimal CTL responses that were easily controlled by transient immune suppression for all four trials (33 combined subjects) reported. By contrast, in three clinical trials, a codon optimization approach to enhance transcriptional and translational efficiency of the expression cassette which increased the number of CpG motifs by approximately fivefold over wild-type cDNA resulted in a strong CTL response that was uncontrollable even with high-dose immune suppression. In the two studies that published outcomes, a complete loss of FIX transgene expression was reported for all but 1 of 14 subjects. Clearly, CpG dinucleotide motifs in the AAV vector can trigger activation of a CTL response and therapeutic transgene loss in human clinical trials of AAV gene therapy, and vector design strategies to decrease the number of CpG motifs in the vector expression cassette are imperative to gene therapy clinical success. Partner with NxGEN Vector Solutions to apply NxGEN Technology to your AAV vectors and eliminate the immune response that limits transgene durability. Contact us at partnership@nxgenvectorsolutions.com. ■

For more information visit:
www.nxgenvectorsolutions.com

NxGEN VECTOR SOLUTIONS

TRANSFORMATIVE TECHNOLOGY

NXGEN TECHNOLOGY OVERCOMES THE CHALLENGE TO EFFECTIVE GENE THERAPY

AAV vectors built using NxGEN Technology can deliver their healing power without activating the body's immune defenses.

Partner with us to apply NxGEN Technology to your AAV vectors and eliminate the immune response that limits transgene durability.

NxGEN VECTOR SOLUTIONS

LEARN MORE AT WWW.NXGENVECTORSOLUTIONS.COM
CONTACT US AT PARTNERSHIP@NXGENVECTORSOLUTIONS.COM

Fast-Tracking Cell Therapy

Manufacturing across Borders

The idea of a borderless world can suggest vulnerability—or the benefits of freely sharing ideas and, ultimately, creating and sustaining globe-spanning cell therapy supply chains

By Josh P. Roberts

The Cell Therapy Manufacturing Asia 2020 conference was held in Kyoto in February, not long before the coronavirus epidemic led to the cancellation of many such gatherings around the globe. Attendance was diminished, and many attendees resorted to teleconferencing. Still, the event provided presenters and attendees an opportunity to share perspectives on a wide swath of topics, from scale-up and process analytics to regulatory navigation. Several representative presentations—some of which were delivered on site, and some of which were live streamed from elsewhere—are summarized in the text that follows.

Vertical scale-up

Cell therapy production for many preclinical studies and some early-stage clinical studies can be accomplished in two-dimensional planar flasks or spinner flasks. Nonetheless, if commercial success is to be achieved, said Brian Lee, PhD, president of **PBS Biotech**, the producer must scale up manufacturing while maintaining the quality of the product as well as the cell growth. "Single-use bioreactors, he added, "are considered a much more cost-effective and scalable manufacturing platform for cell therapy products."

Anchorage-dependent human stem cells cannot grow freely as single cells in the suspension cultures typically used for *Escherichia coli* or adapted Chinese hamster ovary (CHO) cells. Instead, they are grown on microcarrier beads or as cell aggregates while suspended in a bioreactor. Unfortunately, a traditional horizontal, stirred-type bioreactor can pose problems. "As the vessel size increases," Lee noted, "the fluid dynamic characteristics are difficult to scale up while maintaining the conditions at the small scale."

> "If commercial success is to be achieved, the producer must scale up manufacturing while maintaining the quality of the product as well as the cell growth."
>
> —Brian Lee, PhD, PBS Biotech

He asserted that the geometry and mixing mechanism of PBS Biotech's Vertical Wheel™ bioreactor system is "completely different," providing homogeneous mixing and uniform particle suspension in a very low sheer stress environment for cells. The same mixing parameters developed in a 100-mL working volume can be applied to larger-scale vessels to give a similar fluid dynamic microenvironment.

Lee shared data selected from PBS Biotech customers that had used 0.1-L (and above) bioreactors. The company has more than 100 such customers. Although they used different cell types, cell lines, media, and processes, their data, Lee asserted, "demonstrate the difference in the biological performance purely based on the bioreactor fluid dynamic function."

Consistency of scale-up results has been shown at up to 50 L in an 80-L bioreactor, with plans for up to 400- or 500-L vertical bioreactors—sufficient for large-scale commercial manufacturing.

Industrialized MSC supply chain

But does a company even have to source its own stem cells and maintain its own banks? Mayasari Lim, PhD, bioprocessing specialist at **RoosterBio**, argued that it may be better to industrialize this part of the supply chain "so that these companies in the cell and gene therapy space can really start focusing on developing their final products."

Mesenchymal stem cells (MSCs) are adult stem cells that can be used in a variety of ways, for example, as pluripotent or differentiated cells; as engineered tissue, possibly genetically modified for gene therapy; or even as factories for producing extracellular vesicles (EVs).

Providing off-the-shelf cellular starting material—MSCs and optimized bioprocess media—supports RoosterBio's "mission to fuel the rapid commercialization of scalable regenerative cures," she said. The growth medium is formulated so that it doesn't have to be changed every two or three days—typical

in the stem cell field—which is labor intensive and costly. Instead, a nutrient supplement is added to the continuing culture for a fed-batch protocol. For harvesting EVs, RoosterCollect-EV medium is formulated with a low particle count for minimal background.

RoosterBio aims to help companies significantly shorten their clinical development timelines. "They are able to reference our master files that are already with the U.S. Food and Drug Administration," noted Lim. "That reduces the regulatory burden and the cost. By providing them all the cells, all the media, all the starting materials, and by supporting them for their IND filing, we can help them develop the target product that they're going after."

Does potency predict efficacy?

Rohto Pharmaceutical, best known for its over-the-counter pharmaceuticals and cosmetics, is moving into the field of regenerative medicine. It is conducting Phase I/II studies in Japan using allogeneic MSCs as therapeutics for indications such as liver cirrhosis.

It can be frustrating not to know, while developing a cell therapy, if the process will yield the hoped-for product for release. "Potency tests need to predict clinical efficacies," said Hidenori Nonaka, group leader of Rohto's division of regenerative medicine. The potency test should be based on the mode of action. However, whether potency tests will accurately predict clinical efficacies may not be discovered until the late clinical stage or later.

"We know there are some parameters that can differ from one lot to another," he continued. "But we are not sure these differences are really critical to having an effect in the patient. The challenge is, we don't know much about the mode of action, which remains to be understood." This should become clearer as data comes in from the clinical trials.

Rohto is looking at several different assays that can be performed on the intermediate and final products (cells) or disposed material (such as supernatant) along the way, to help find critical quality attributes that will correlate with a successful product. The company is focusing on potency tests for matters such as immunomodulatory function, differentiation capability, and senescence of the MSCs as part of a matrix of complementary measures.

Raman as PAT

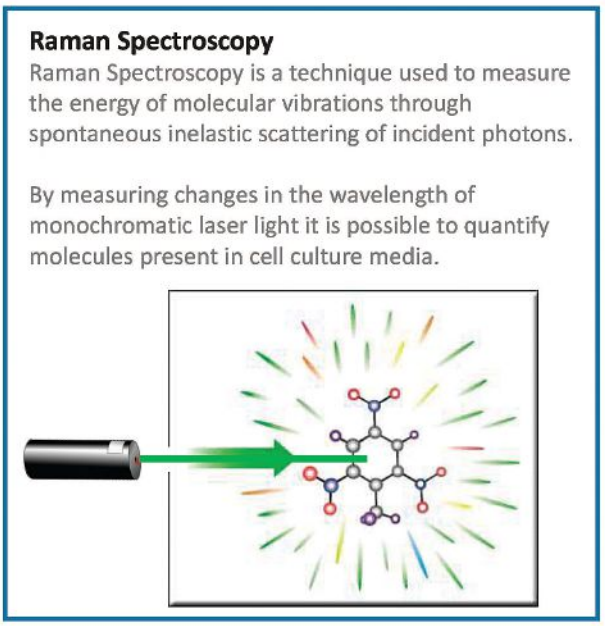
Even when you know what a healthy culture should look like, it's not always straightforward to gather information about the process in a timely fashion. "It's not until you get to the end of the manufacturing process that you can actually understand how successful that process has been," remarked Damian Marshall, PhD, director of new technologies for the Cell and Gene Therapy Catapult.

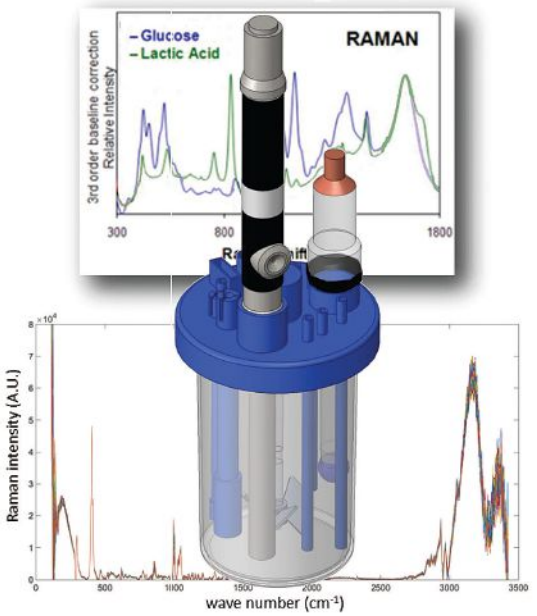
Part of the delay is due to the dearth of process information technologies (PAT)—which use timely measurement of critical process attributes to ensure final product quality—in cell and gene therapy manufacturing. "Typically," Marshall said, "it has been retrospective."

Marshall wanted instead to interrogate the processes that were indicative of final quality, however defined. They chose 12 metabolite markers that were expressed in the media at key points within the manufacturing process, when changes were occurring, and developed Raman spectroscopy models to detect what was happening with those markers within the culture environment. "And then we did a proof-of-concept demonstration where we actually looked at tracking all of these markers in real time within the system."

Raman, a technology that can be deployed in real time, was chosen as a starting point rather than another technology that would involve "taking samples and doing an offline analysis," he explained. It's much less affected

At the Cell and Gene Therapy Catapult, analytical scientists use Raman spectroscopy to monitor changes in nutrient consumption and metabolite production during the bioprocessing of cell therapies. Applying a univariate modeling approach, the scientists can correlate changes in peak intensity within Raman spectra with cell concentration and viability.





Fast-Tracking Cell Therapy Manufacturing across Borders

by the signal that comes back from water in the culture medium than are near- or mid-IR spectroscopy. "We also wanted a technology that had been demonstrated in a GMP environment," Marshall noted.

The next step, Marshall indicated, is to use the PAT information for making processing decisions: "It's the concept of adaptive manufacture."

Mini-PAT

As the field moves forward, there is little question of the utility of PAT for process monitoring and quality control. "[But identifying] tools that are fast, precise, robust, and cost-effective is still a challenge," observed Katleen Verleysen, PhD, strategic partnerships life sciences, IMEC.

IMEC focuses on digital and nanoelectronics, developing technologies that manufacturers can incorporate into their own processes. "We get to play around with silicon chips," she remarked.

Silicon is flexible, easy to parallelize, and disposable. "You can have all the necessary functionalities incorporated on your disposable so that you don't have to have these in your instruments," she elaborated. "I'm talking about the electronic boards, all the optics, and everything."

Verleysen presented concepts such as miniaturized liquid sensors, on-chip Raman spectrometers, lens-free imaging microscopes, and rapid contaminant detection and identification. And these disposable chip-based technologies are not restricted to PAT. For example, IMEC has developed a cell sorter that can do label-free sorting based on a holographic image, obviating the need for magnetic beads or fluorescent tags, which have the potential to contaminate the sample. These technologies are either currently available or under development.

She hopes that miniaturization, parallelization, and multiplexing of these functionalities can reduce the need for sampling, open possibilities for continuous monitoring of multiple parameters, and lead to better quality control.

Geography matters

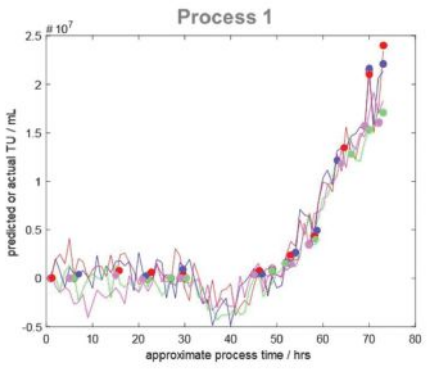
Most cell therapies—including CAR T-cell therapies, for example—are autologous, derived from the patient. Among the appeals of an allogenic cell therapy, on the other hand, is that the starting material can be obtained from unrelated donors, meaning that the final product need not be restricted to the donor from whom it was derived. This allows for a potentially unlimited supply to be generated in bulk and then stored in individual doses as an essentially ready-to-use off-the-shelf product.

Antonio S.J. Lee, PhD, global head of business development for **Medipost**, a Korean stem cell therapy company, and CEO of the company's **Medipost America** subsidiary, discussed the challenges of navigating the regulatory environments of distinct geographical regions. He highlighted Medipost's experience bringing an allogeneic stem cell product to market in Korea. The product, called Cartistem®, incorporates mesenchymal stem cells derived from human umbilical cord blood. It was approved by South Korea in 2012 for the treatment of cartilage defects such as osteoarthritis and has been engrafted in over 13,000 patients to date with no adverse events reported.

Medipost has completed Phase I/II studies in the United States and Japan, and it is currently planning Phase III trials in the United States, Japan, and China. The company has found that it is important to consider the local clinical practice and regulatory stance when devising a clinical development strategy and study design.

"The requirements and expectations regarding safety and efficacy of each batch of manufactured cell therapy batches are slightly different between regulatory agencies," Lee commented. Recent meetings with regulatory agencies in Japan and the United States both "provided positive feedback on our proposed Phase III clinical trial design," he noted, but he was unable to elaborate on the technical details due to confidentiality concerns. GEN

	Marker	Function	Raman Mean R^2
1	Proline	Amino acid	0.94
2	Glucose	Glycolosis	0.84
3	Hydroxyphenyl lactate	lactic acid	0.89
4	Asparegine	Nitrogen donor	0.90
5	Histidine	Amino acid	0.82
6	Spermine	Cholesterol metabolism	0.58
7	Malate	TCA cycle by-product	0.92
8	Glutamine	Amino acid	0.83
9	Glycine	Formation of Serine	0.92
10	Lactate	Glycolosis by-product	0.92
11	Serine	Amino acid	0.88
12	Pyruvate	Glycolosis	0.87



Scientists at the Cell and Gene Therapy Catapult selected 12 metabolite markers and developed Raman spectroscopy models to enhance the monitoring of cell culture during product manufacture. Ultimately, through the application of advanced process analytical technology, real-time monitoring should enable dynamic feedback control of bioprocessing.

NEW

Improve your residual endonuclease assay with EndonucleaseGTP ELISA from Cygnus Technologies!

- The most sensitive endonuclease ELISA kit: LOD ~0.06 ng/ml; LLOQ ~0.31 ng/ml
- Suitable for the detection and quantification of genetically engineered endonuclease from *Serratia marcescens*
- Qualified for assay precision, sensitivity, and accuracy
- Fast protocol with results in <2 hours
- Seamless transfer from purification process development to QC testing and lot release testing

Cygnus Technologies continues to support your gene therapy and vaccine commercialization projects with industry-leading host cell protein ELISA kits for most viral vector and vaccine production cell lines:

•HEK293 •Sf9 •BHK •HeLa •A549
•MRC5 •PER.C6® •CAP® •Vero •MDCK

Trust your assays. Trust your results.

Learn more at: **cygnustechnologies.com/endonucleaseGTP**

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

The Right Tools to Optimize Cell Therapies

Challenges and opportunities for efficient process development

The global market for cell therapies just keeps growing. Whether it's CAR-T cells for cancer therapy, gene therapy applications, or another infusion of living cells, developing cell products for commercial therapies poses novel challenges in all aspects of development, testing, and manufacturing.

With a living cell product, safety testing, regulatory requirements, and large-scale manufacturing cover uncharted territory. Autologous products, which are made to order in small batches from patient cells, are labor intensive to produce. Allogeneic therapies intended for off-the-shelf use require different methods of testing and regulatory standards than conventional small molecule drugs.

"Each cell therapy is unique, they're all case-by-case," says Shawna Jackman, PhD, senior principal research scientist with Charles River Laboratories. We're able to support these programs by leveraging our expertise in different areas to provide guidance and tools to advanced medicine researchers."

Start strong, finish strong

Choosing the appropriate starting material can go a long way toward smoothing the path to a successful final product. Yet the starting material is too often a source of great variability when it comes to cell production. To meet regulatory quality standards, working with a trusted supplier to provide consistent, high-quality cells is essential.

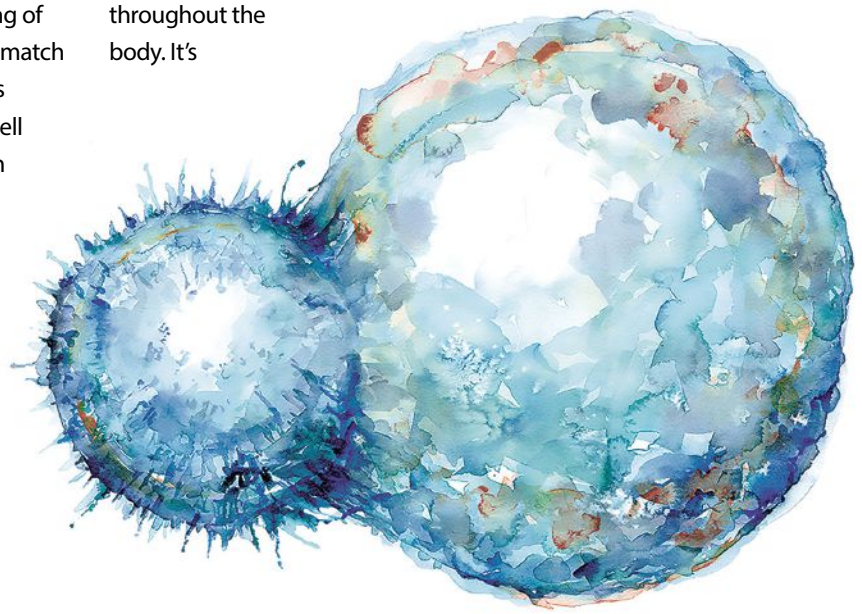
Access to a large and diverse donor pool allows for close matching of characteristics to the criteria needed in the final product. "If you can match early, you save valuable resources when transitioning to later phases of process development," says Dominic Clarke, PhD, global head of cell therapy at HemaCare. For instance, he says, it can be useful to screen donors by HLA type, disease state, or age. Whether for allogeneic or autologous products, matching donor characteristics can be a critical but easily overlooked step in building a consistent and efficient manufacturing process.

The perfect balance: Safety and efficacy

Once the candidate therapy is engineered from the starting material, it's time for lead optimization. "Different versions of a cell therapy are compared to each other, in terms of functionality, potency, and cross-reactivity in different bioanalytical tests," says Sanne Holt, PhD, group leader at Charles River Discovery. Using CAR-T cells as an example, she points out that while selecting for higher potency seems desirable resulting in higher cancer cell death, cells with the highest potency also pose the highest potential safety risks. "The in vitro efficacy tests that you perform first will help identify that optimal lead candidate," she says. "Is the best candidate also the safest candidate? Ideally you need to find a perfect balance where it's safe and still potent enough."

Following efficacy testing, in vitro safety testing aims to identify risks to normal healthy tissues through potential off-target effects of these therapies. "You select human tissues of suspected risk, either tissues exhibiting low-level expression of the target antigen or major organs which need to be evaluated," Holt says. Co-culturing the lead candidates with healthy primary human cells, and verifying there's no response, provides a scientific rationale that the therapy will work as intended without causing unintended havoc around the body.

Once several promising candidates have made it through in vitro testing, they are evaluated in animal models to further demonstrate their safety and efficacy. "Cell therapies possess unique attributes, and evaluating these products can be different from the traditional approaches to drug discovery," says David Harris, PhD, research director at Charles River Discovery Services. "These therapies can replicate and persist throughout the body. It's



important to understand how they will behave in animal models before they are tested in humans."

As far as possible, the cellular therapy being tested in animals should mimic the attributes of the final clinical grade product, to accurately evaluate the potential functionality in patients. Using the example of CAR-T cells, Harris explains, the cells are engineered to express a receptor that binds to an antigen on a target cell. But if the target antigen is also expressed on healthy tissues, there can be unintended and potentially serious responses. "There can be life-threatening consequences of engaging these highly activated cytotoxic T cells in certain situations," Harris says. The in vivo pharmacokinetic studies help identify potential side effects, allow researchers to determine the optimal dose and provide critical information about the pharmacodynamic properties of the therapy. "Generally, one is trying to find that sweet spot of robust activity with the optimal number of cells."

Utilizing an appropriate animal system is also critical. The preclinical models should express the specific target and represent the tumor type that will be treated, Harris points out. "We utilize a wide variety of different tumor model systems depending on the nature of the cell therapy evaluated."

These studies play a critical role in establishing efficacy and evaluating safety that are required for regulatory approval. Here again, cell therapies push manufacturers into uncharted waters when it comes to drug development.

Traditionally, regulatory agencies require that studies are conducted according to GLP guidelines. However the dynamic systems involved in assessing living cell therapies mean it's not always possible to adhere strictly to GLP guidelines. "In these exceptions, all the components needed for reliable, robust, and reproducible data are still incorporated," Jackman says. "We work with clients to navigate those specifications and present the high-quality science necessary to meet regulatory expectations."

Process optimization and scale up

Once efficacy and safety have been established, it's time to start manufacturing. "The goal is not only to develop a safe product, but to develop a consistent and repeatable process," says Clarke. The path will be slightly different for autologous products from disease patients than for allogeneic products made from healthy donor cells.

For development of an autologous product, establishing a well-defined process is difficult given the inherent patient-to-patient variability. Ideally, the disease-state starting material would be used, but sourcing large amounts of cells from sick donors represents a key hurdle. Due to the sourcing limitations, starting material from healthy donors is used. In order to develop a process that can accommodate the variability, it's important to work with heterogeneous donor material, like a leukapheresis collection, because that will resemble more closely what's collected from the patient. "Working with the right starting material during process development is critical as it enables you to gain downstream processing proficiency," Clarke says. "Ultimately, you're building a consistent manufacturing process that's going to allow you to characterize and deliver a final product that consistently meets the target product profile."

With allogeneic or off-the-shelf products, establishing safety and consistency of the donor-derived starting material becomes more critical. Having access to an extensive, highly characterized donor pool for selection and sourcing is important for initial process development and long-term supply continuity. Cells from certain donors might have characteristics that allow for more efficient transduction or faster cell expansion rates. "There's ultimately both internal and external variability associated with the donors, and that variability impacts how that product is manufactured downstream," Clarke says. "One of the critical components we provide is access to recallable donors, which are donors that have demonstrated longstanding commitment and are reliable." If certain donor cells work best, having access to additional collections from those same donors provides a significant benefit to manufacturers by reducing variability and establishing process and product consistency.

Ultimately, the donor starting material in support of allogeneic therapies will have to be collected and manufactured to cGMP (current Good Manufacturing Practice) compliant standards for clinical application. "This is something we work with our clients on through early and active collaboration," says Clarke. "Not only can we collect the cells but with onsite GMP-compliant cleanrooms, we can support some of the key manufacturing steps including cell isolation and cryopreservation necessary to support clinical and commercial production."

Delivering the final product

The GMP release and characterization testing that is applied to cell therapies follow the basic tenets for all biologic drugs: sterility, mycoplasma, endotoxin, viability, identity, and potency assessments. Many of these tests need to be performed using a rapid testing platform, as the time from harvesting the cell therapy to dosing the patients can be relatively short. Collectively, this testing provides end-to-end documentation that assures the safety and efficacy of the production of the cell therapy products.

The safety risks for each product and process can vary with each type of cell therapy, but the overarching goal is to provide consistent and effective therapies and specific testing strategies that ensure patient safety. Partnering early is an important aspect to creating the support and continuity necessary for success. It is good to remember that with new therapies come new challenges—these are exciting times. ■

Learn more about Charles River
https://bit.ly/2RTIEXd

charles river

Gene Therapy Developers

See an Expanded Viral Toolbox

Better analytical tools, production processes, and scale-up approaches are making it easier and more cost-effective to build viral vector–based gene therapies

By Mike May

Gene Therapy Analytics and Manufacturing, a recent conference organized by the Cambridge Healthtech Institute and held in San Diego, described itself as a way to take "an in-depth look at the challenges facing the formulation, characterization, analysis, and scale-up of gene therapies." The conference lived up to its promise, and it was, as this article maintains, particularly strong in its coverage of viral vectors. In the following text, five presenters from the conference offer their views on viral vector–based gene therapies. (Readers may notice that this conference was, along with the Cell Therapy Analytics and Manufacturing conference, part of the Cell and Gene Therapy "pipeline" at PepTalk: The Protein Science Week.)

Combatting complexity

"Viral vectors are complex structures of several megadaltons, consisting of nucleic acid and a protein shell," said Klaus Richter, PhD, group leader and analytical ultracentrifugation (AUC) expert at **Coriolis Pharma**. "In some viral species, a membrane is additionally present with proteins embedded in it."

To make a viral-based therapeutic, all the parts of the virus must be functional. "This is very different from small molecules or classical biopharmaceuticals, where the administered drug substance already contains the actual active agent," noted Richter. "In addition, the whole structure needs to be stable because the viral vectors need to maintain their ability to infect cells." These requirements make quality control a very complex activity.

To assess the quality of a virus, such as an adeno-associated virus (AAV), explained Richter, scientists can use sedimentation velocity (SV)-AUC. For example, SV-AUC can be used to analyze the DNA in the protein shell, the percentage of empty or partially assembled viruses in an AAV preparation, and the percentage of aggregated AAVs. Richter added, "These are critical parameters to confirm the quality of the final product and to assess its stability and shelf lifetime."

Manufacturers can add SV-AUC to their existing process, Richter observed, "provided that enough time is allowed for performing the experiment and data analysis, which together can be a few hours using optimized procedures for AUC analysis and data handling." According to Richter, this method can be used in development to detect viral particles at particular steps in manufacturing, for example, to evaluate enrichment or on a final product to determine if the "critical parameters for quality control are met."

Swapping out silver staining

The detection of AAV capsid proteins can be used to assess the quality of the particles. Often, scientists perform this detection with silver staining of sodium dodecyl sulfate-poly-

Coriolis Pharma, a contract research organization that specializes in formulation development of biopharmaceuticals, performs sedimentation velocity ultracentrifugation (SV-AUC) experiments to assess the quality of adeno-associated virus preparations. Pertinent measurements include the relative amounts of differently sedimenting species (filled and empty capsids).

acrylamide gel electrophoresis (SDS-PAGE), but scientists may try alternative techniques. For example, as a presentation from **PerkinElmer** demonstrated, scientists may use capillary electrophoresis-SDS (CE-SDS).

"The AAV capsid is composed of three proteins, which are designated as VP1, VP2, and VP3," said James Atwood, PhD, general manager of applied genomics at PerkinElmer. "The proteins differ in size with reported values of 87, 73, and 62 kilodaltons for VP1, VP2, and VP3, respectively." In producing a recombinant AAV, a manufacturer must analyze the distribution, size, and ratio of the viral capsid proteins. Atwood noted that this quality-control step is used "to validate capsid protein expression, to screen for protein contaminants, and to verify assembly of the capsid in the appropriate stoichiometry."

At the conference, PerkinElmer discussed a microfluidic CE-SDS method for characterizing capsid proteins. "AAVs are diluted in a nonreducing sample buffer and heated to dissociate the viral capsid into individual protein constituents," Atwood explained afterward. "Standard protocols of the LabChip ProteinEXact Assay are then followed for preparing the LabChip assay with a gel and dye solution." The samples are analyzed on the LabChip GXII Touch instrument, which automatically analyzes the AAV protein capsid size for each sample.

"A typical silver staining SDS-PAGE experiment takes approximately 60 to 120 minutes, but it only takes approximately 60 seconds for each sample run on the LabChip system," Atwood asserted. "The LabChip ProteinEXact Assay can detect protein concentrations as low as 0.2 nanograms per microliter, which is about an order of magnitude lower than silver staining SDS-PAGE."

Overall, Atwood described this approach as "a fully automated and validated solution delivering quantitative and reproducible digitized results to monitor the quality of AAV particles."

The PerkinElmer LabChip GXII Touch, an instrument for performing protein and nucleic acid analysis, can distinguish differences of protein molecular weight as low as 1 kDa. By reproducibly and accurately measuring capsid composition, the instrument can facilitate the characterization of recombinant viral particles, a crucial step in gene therapy applications.

Making more use of MVM

Analyzing a biopharmaceutical process for its ability to clear virus, said David Cetlin, founder and CEO of **MockV Solutions**, "requires spiking with a live infectious agent—virus." He added that contract research organizations (CROs) "are typically the only avenue for conducting viral clearance work," which is often so costly and complex that it is limited to late-stage validation. So, Cetlin and his colleagues created the first viral clearance prediction kit.

In this kit, Cetlin explained, a noninfectious "mock virus particle" (MVP) replaces the usual live, infectious virus. He added that the MVP "mimics the physicochemical characteristics of MVM (minute virus of mice), a parvovirus commonly used for spiking studies." This so-called MVM-MVP system offers a range of benefits, starting with the ability to use it in biosafety level 1 (BSL-1) conditions.

"Whereas live MVM requires a BSL-2 laboratory, MVM-MVP can be used on any common benchtop, thereby enabling studies to be performed on site, as opposed to on location at a CRO," Cetlin asserted. "The MVM-MVP kit, which contains a stock solution of MVM-MVP, is also much cheaper than contracting a CRO-led study." At $4000 per kit, which can run about 10 small-scale studies, this method only costs about $400 per experiment. Cetlin added, "Sample and data analysis can also be conducted in a matter of hours, as opposed to the weeks that would be required for a plaque assay readout at a CRO."

In his talk, Cetlin described the physicochemical similarities between MVM and MVM-MVP, explained how to conduct an experiment utilizing the MVM-MVP, and showed data sets from three collaborations in which MVM-MVP was used in process development/characterization efforts. "The data not only demonstrated that MVM-MVP can predict MVM's removal within an AAV process, but also demonstrated that **Thermo Fisher's** AAVX resin can distinguish between AAV and MVM, which are both parvoviruses," he stated. An MVM-MVP

Wyatt Technology, a specialist in dynamic light-scattering instrumentation, has demonstrated that a combination of size-exclusion chromatography, a multiangle light-scattering platform, and ultraviolet and differential refractive index detectors can be used to assess the quality of adeno-associated virus gene therapy vectors.

Gene Therapy Developers See an Expanded Viral Toolbox

study, he concluded, can serve "as a good viral clearance reduction step in an AAV process."

Learning more from light scattering

Developing gene- and cell-based therapies requires methods for characterizing and quantifying AAV and other viral vectors. Michelle Chen, PhD, vice president of analytical services at **Wyatt Technology**, discussed how light scattering can be used in those processes with three tools: dynamic light scattering (DLS) for fast screening of viral vector size distribution and particle concentration; size-exclusion chromatography (SEC) combined with multiangle light scattering (MALS) for some crucial AAV quality attribute measurements; and field flow fractionation (FFF) combined with MALS for characterizing AAV aggregates, large viral vectors, and other bionanoparticles.

"Our SEC method, coupled with UV, MALS, and differential refractive index (dRI) detectors, is used to measure three critical quality attributes of an AAV product—total capsid concentration, capsid content, and degree of aggregation—within one single SEC run," Chen said. She noted that this method requires no calibration or label, runs in 30 minutes or less, and is fully automated. Chen added that it is "robust and has the potential to be implemented in AAV manufacturing, quality control, and quality assurance."

Using the combination of different detectors in Wyatt's protein conjugate-analysis method provides the "molecular weight and eluted mass for the protein capsid and DNA," Chen detailed. "These quantities can then be readily converted to capsid-particle concentration and empty-to-full ratio." Plus, she noted that "the data from the same run can be used to quantify aggregation, detect impurities, and measure particle size."

Results indicate the benefits of this method. "Compared to the other techniques," Chen asserted, "the SEC-MALS tool provides an orthogonal and complementary approach with easy implementation and validation throughout the AAV production process."

Batavia Biosciences, a contract development and manufacturing organization, is part of a consortium led by the Leiden University Medical Center and focused on developing a lentiviral vector–based therapy for severe combined immunodeficiency syndrome (SCID). In this consortium, Batavia is applying its process development and manufacturing expertise.

Looking at lentiviral vectors

Severe combined immunodeficiency syndrome (SCID) arises from a genetic defect that prevents the development of the adaptive immune system and leads to "a wide range of life-threatening infections like pneumonia, meningitis, and sepsis," said Alfred Luitjens, director cell technology, **Batavia Biosciences**. "Babies with SCID die within their first year."

SCID is associated with 20 or more genes. RAG1-SCID, a common form of SCID associated with a RAG1 variant, is the focus of a consortium led by the Leiden University Medical Center (LUMC). As a partner in the consortium, Batavia is developing good manufacturing practices for the production of a lentiviral vector–based therapy.

"One of the next steps in this project will involve bringing the process to commercial manufacturing scale with the aim to treat all babies with RAG1-SCID worldwide," Luitjens said. For this process, Batavia developed a model that uses **Univercells**' scale-X bioreactors and NevoLine biomanufacturing system. The consortium hopes to develop "an autologous transplant system," Luitjens noted. "The patients' own blood-forming stem cells will be collected and sent to the transduction site, the LUMC in the Netherlands. Then the modified stem cells will be returned to the clinical centers and transplanted into the patients."

When asked to summarize the process, Luitjens said it involves "getting the blood-forming stem cells in good and consistent condition at the LUMC, performing the transduction, and subsequently transporting the genetically modified stem cells back to the treatment center." If the results of the Phase I study are encouraging, the process will be scaled up with the scale-X bioreactor. GEN

APPLIED GENOMICS

QUANTITATIVE, HIGH-THROUGHPUT AAV CHARACTERIZATION

LabChip® GXII Touch™ Protein Characterization System

The Quantifiable Alternative to SDS-PAGE with Silver Stain

The LabChip® GXII Touch™ protein characterization system is an automated research platform that offers unparalleled potential to quantifiably study adeno-associated virus (AAV) proteins. Combined with LabChip® ProteinEXact™ assay, this platform provides high-throughput standardized characterization of AAV proteins.

Visit PerkinElmer-AppliedGenomics.com/AAV to improve the efficiency, accuracy, and reproducibility of your AAV characterization.

- Efficient, accurate, and reproducible
- Measures up to 384 samples in one instrument preparation
- Uses 2 µL of sample
- Analyzes one sample within 65 seconds

PerkinElmer®
For the Better

For research use only. Not for use in diagnostic procedures.

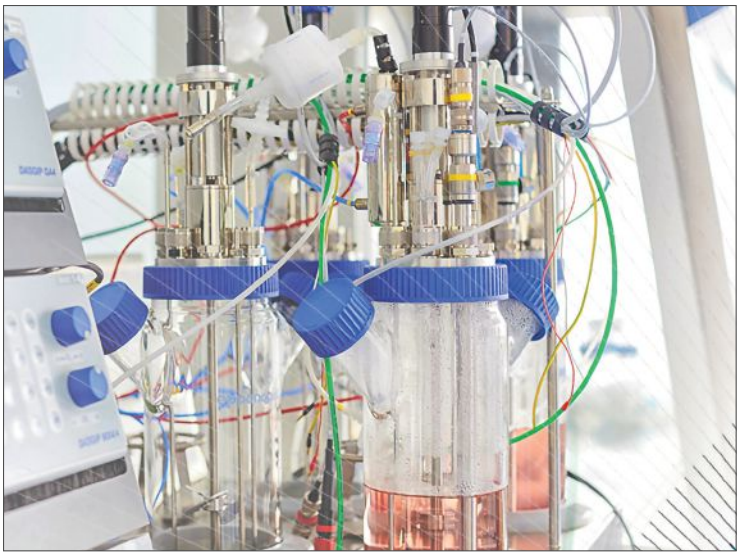
Gene Therapies
A History of Transformative Technologies

The history of gene replacement therapies is a tale of scientific endeavor, persistence in the face of adversity, and world-changing discoveries. It encompasses breakthroughs in cell biology, molecular biology, biochemistry, structural biology, immunology, virology, oncology, engineering, and biotechnology. Yet despite the theoretical simplicity—overriding the disease-causing effect of a missing or faulty gene by inserting a working copy—there are still very few gene replacement therapies on the market today, 30 years since Rosenberg *et al*. demonstrated the potential of retroviral based gene transduction in humans (Rosenberg *et al*. *N. Engl. J. Med.* 1990; 323: 570–8).

Boom: The roaring nineties

The early 1990s were halcyon days for gene therapies. Researchers and clinicians alike believed that they held the key to curing all genetic diseases. Academics, investors, start-ups, and spinouts scrambled to enter this promising new market, driven by the hope of developing revolutionary treatments for gene-based disorders.

At this time, most gene therapy trials used adenoviruses to deliver the transgene into patients, a technique made possible by Professor Frank Graham's work in the early 1970s to understand why some viruses are oncogenic, while others aren't. In 1973, Graham—then a postdoc at the University of Leiden in the Netherlands—successfully created an adenovirus-transformed immortal human cell line, Human Embryonic Kidney (HEK)293 (Graham *et al. J. Gen. Virol.* 1977; 36(1): 59–74). HEK293 cells are easily transfected and contain the adenoviral E1 genes, which allow replication-incompetent adenoviruses to continue to grow in these cells. These characteristics make them an obvious choice for producing the large quantities of viral vector required for a human gene therapy.



While gene therapy was booming, the human genome project, another remarkable feat of scientific investigation, was also underway. Fifteen years of global research collaboration resulted in the first publication of the complete human genome sequence in 2003. Scientists now had access to not only the sequence of every human gene, but also maps detailing the location of these genes within chromosomes, and linkage maps to track the inheritance of genetic disease (*Science* Apr. 11, 2003 and *Nature* Apr. 24, 2003, full issues).

Success, stall, repeat (2003–2017)

With such a wealth of information available, it's unsurprising that the gene therapy industry persisted in its attempts to revolutionize modern medicine.

In 2003, the China State Food and Drug Administration became the first health authority in the world to approve a gene therapy—an adenoviral vector carrying the P53 tumor suppressor gene—called Gendicine. However, we had to wait until 2017 before the U.S. Food and Drug Administration (FDA) approved the first gene therapy—Luxterna, for retinal dystrophy—for use in the United States (source: genetherapy.net).

Yet despite decades of research and investment, the scarcity of gene therapies currently on the market—and the cost of those that made it this far—speaks to the challenges still facing their development and manufacture. In particular, the challenge of cost-effective manufacture at the required speed, scale, and quality for clinical development remains to be overcome. For gene therapies like Luxterna, which target localised diseases and require only small doses, manufacture is relatively simple.

However, the publication of results from a successful hemophilia gene therapy trial in 2011 not only reinvigorated the gene therapy industry, but highlighted the need for new, scalable technologies to support the manufacture of gene therapies for systemic diseases that require high treatment doses (Nathwani *et al. N. Engl. J. Med.* 2011; 365: 2357–2365).

A look to the future: Transformative solutions to manufacturing challenges

At present, most gene therapy manufacturers rely on "scaling out" transient expression platforms. This is both costly and resource intensive, due to the large amounts of GMP-grade plasmid DNA required

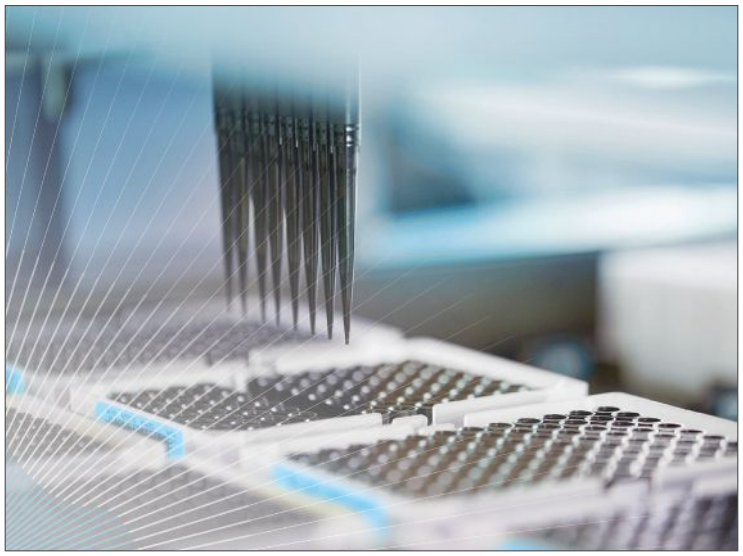
and/or the enormous cell culture footprint demanded by adherent cell cultures. But the future of gene therapy vector production undoubtedly lies in stable manufacturing solutions that can be easily "scaled up."

At OXGENE™, our established expertise in DNA design and engineering, cell line development, upstream and downstream processing, and industry-leading automation is driving the transformation of our optimized AAV and lentiviral transient expression platforms toward alternative technologies for scalable, stable manufacturing.

We have strong foundations upon which to build. Our gene therapy production platforms are founded on proprietary SnapFast™ plasmid technology. These are modular plasmids, designed to work like "molecular Lego™," using a catalogue of characterised DNA elements that can be easily and reliably inserted into specific locations within the plasmid. Our engineered AAV and lentiviral plasmids significantly improve packaging efficiency and viral titre, while our clonal HEK293 suspension cell line was specifically selected for optimal viral vector production (view data at: www.oxgene.com/News-and-Events/Gene-Therapy-Posters).

Joining forces with OXGENE in the early stages of gene therapy development allows our partners to establish and optimize transient production, including validated production up to 10-L scale, before transitioning to a stable technology platform for large-scale clinical manufacture. This provides the additional regulatory advantage of using the same genetic system throughout clinical development, as the stable platform retains the same expression cassettes and base cell line as the transient system.

Producer cell lines are an attractive alternative to transient transfection. Here, all the elements required for viral vector production, as well as the transgene of interest, are stably integrated into the cell's genome. They therefore require no transfection and relatively little manipulation to scale up and consistently produce large quantities of viral vector, with lower batch-to-batch variation and at significantly lower cost. We've now successfully developed stable packaging and producer cell lines for lentivirus-based gene therapies.



We generated a stable lentiviral packaging cell line by transfecting packaging plasmids reconfigured with inducible vsv-g and gag-pol and constitutive rev expression into our HEK293 cell line. We then screened single-cell clones for growth kinetics, as well as stable—and inducible—expression of the viral genes. After several rounds of testing and analysis, we selected a single clonal lentiviral packaging cell line to expand, characterize, and optimize further. Process optimization has so far improved viral titer more than 10-fold.

The high level of optimization involved in perfecting OXGENE's lentiviral packaging cell line makes this an excellent starting point from which to generate producer cell lines by stably transfecting a transfer plasmid containing a self-inactivating lentiviral genome and the transgene of interest. After another iteration of the cell line development process, the best-performing clones are expanded further and transferred for process optimization and scale-up to maximize viral titer.

With AAV, we've taken a different approach. We're using our novel Tetracycline-Enabled Repressible Adenovirus (TERA) system as the basis for a stable AAV production platform. This uses an engineered helper adenovirus that contains a switchable negative feedback loop in the viral genome. This reduces helper adenovirus contamination to effectively zero and increases AAV yields. We have also shown that we can use this system to amplify both AAV rep and cap DNA from the cells' chromosomes using the well established AAV Cis-Acting Replication Element (CARE). This technology allows the stable integration of DNA into cells, its subsequent amplification, and concomitant high protein expression levels, which provides a scalable, stable, and adenovirus-contaminant-free AAV manufacturing process.

Conclusion

Gene therapies are poised once again to transform the treatment of some of the world's most devastating diseases. However, manufacturing challenges to date have hindered their development and approval. OXGENE's ambition is to transform gene therapy manufacturing. By pioneering the development of tightly controlled and carefully optimized technologies to enable fully scalable, cost-effective, and high-quality gene therapy manufacture, OXGENE will help bring gene therapies to the patients who need them. ■

To learn more visit
www.oxgene.com

OXGENE™

Cell Therapy Manufacturing

Processes as Individual as the Products

If every process development project poses a unique set of analytical, scaling, and quality control challenges, every developer must find a unique path from lab to clinic

By Catherine Shaffer

Cell-based therapy is progressing quickly, at least in terms of research and development. Important trends include hematopoietic progenitor cell transplantation, autologous therapies for wrinkles and cartilage defects, autologous cellular immunotherapies, and chimeric antigen receptor (CAR) T-cell therapies. In addition, many novel cell therapies are in the development pipeline.

It must be emphasized, however, that cell-based therapies are complex. They represent living products. Accordingly, they present unique manufacturing challenges. These include challenges in process scale-up, process analytics, and product characterization. Until these challenges are overcome, cell-based therapies won't receive widespread rollouts.

Initiatives to improve the manufacture of cell-based therapies were highlighted at PepTalk: The Protein Science Week. This event, which was held January 20–24 in San Diego, included a "Cell and Gene Therapies" pipeline that delivered world-class presentations about emerging opportunities and persistent hazards in manufacturing and analytics.

Shorten time to therapy

One of the major challenges for cell therapy is that the manufacture of autologous products cannot be scaled up. Unlike traditional biopharmaceutical products, where larger reactors often represent a convenient solution to the scale-up problem, autologous cell therapies are single-batch products from individual patients. One strategy for getting around this difficulty is using therapies based on natural killer (NK) cells rather than T cells.

At the PepTalk event, this strategy was discussed by Sandro Matosevic, PhD, assistant professor, department of industrial and physical pharmacy, Purdue University. He presented his work on the developing CAR NK-cell immunotherapies against solid tumors.

Matosevic said that NK cells have a higher potential to be used allogeneically. That's because they work when there is a human leukocyte antigen (HLA) or major histocompatibility complex (MHC) mismatch. Matosevic says that NK cells look for cells that are a mismatch and kill them, which means the bigger the mismatch, the better it works as a cell therapy. In contrast, T-cell therapies will not work when mismatched. In addition, CAR NK-cell therapies do not lead to graft-versus-host disease (GVHD), which is a major adverse event associated with CAR T-cell therapies in clinical studies.

Typically for autologous T-cell therapies, it takes 3–4 weeks to manufacture the therapy. That's because blood has to be taken from the patient and taken to a facility so that T cells from the blood can be engineered into a therapy, and then shipped back to the hospital where the patient receives it.

"For NK cells, though, this can be shrunk a little bit because we can have off-the-shelf cells. If the cells are already waiting and the patient doesn't have to give blood, they can be ready a little quicker," asserted Matosevic. That's accomplished by using stem cells to create synthetic blood cells in the laboratory.

NK cells engineered to target CD73 have been shown to kill glioblastoma cells, lung cancer cells, and prostate cancer cells in vitro. For glioblastoma, a notoriously immunosuppressive tumor type, targeting multiple pathways with NK cells instead of a single antigen has shown promise.

"Targeting multiple pathways at the same time through genetic engineering is really the way to go for these treatments," Matosevic emphasized. "Targeting just one antigen at the same time like CAR T cells do for leukemia does not work for these tumors."

One manufacturing challenge for NK cells is persistence. T-cell therapies can be persistent in the body for months, leading to a long-lasting immune response. NK cells typically last no more than two weeks. Persistence can be prolonged by infusing the cells into patients with cytokines. According to Matosevic, in a clinical trial, NK cells given to patients with IL-15 had a presence in the body up to or over 12 months.

"It's really an important consideration in terms of their activity when they're given as drugs," Matosevic pointed out. "You do want a higher response than what they're able to sustain, but you don't want them to be active forever."

Exosomes as alternatives to cells

As promising as cell therapies are, they have some disadvantages. Those include issues with efficacy and cytotoxicity, high cost, long wait times, and adverse pharmacokinetics in the body. Exosomes are a potential alternative that can be adapted to deliver many different types of therapies.

In nature, exosomes play a role in communicating signals in the cell as well as transmit-

ting disease. As a therapeutic, exosomes have an advantage over cell therapies in that they do not need to be genetically engineered. Also, exosomes are made of cell membranes rather than synthetic polymers or modified cells. So, exosomes are well tolerated by the host.

Exosomes, however, also present some unique manufacturing difficulties. Their very small size makes them difficult to separate, and they overlap in size and surface chemistry with microvesicles, apoptotic bodies, viruses, and other objects in the cell. They don't tolerate extreme chemical conditions, and their surface chemistry is not uniform, limiting the type of purification processes that can be used.

Wasfi AlAzzam, PhD, CSO at **TechnoPharmaSphere**, presented a novel process for manufacturing exosome therapies. "In cell therapy, you need to separate the cells from patients, and then you need to genetically modify the cells," he said. "Accordingly, the degree to which these immune cells are accepted will vary. They may be attacked by the patient's immune system. Exosomes don't have these side effects, so they can be very good therapeutic agents."

On the analytics side, some useful technologies for detection of exosomes are SEC-MALS (size-exclusion chromatography with multiangle light scattering), SEC-immunofluorescence, and SEC-picogreen. These can provide insights that complement conventional monitoring methods. SEC doesn't discriminate exosomes from non-exosomal vesicles, but it can give a helpful perspective on the overall size distribution of contaminants. Reduction of contaminants can be carried out by tangential flow filtration or commercial kits that precipitate contaminant vesicles.

AlAzzam noted that other size measurements, such as those obtainable via Luminex' Amnis flow cytometry instrumentation, can enable estimation of exosome size, counts, and conservation of immunological integrity. The overall exosome development process, he added, could be scaled up or scaled down for development, validation, and manufacturing.

Meeting supply demands

Kelly Kemp, PhD, director of process development at **ViaCyte**, offered an overview of her company's work scaling up cell-based processes for clinical trials. ViaCyte is developing islet replacement products that are based on pluripotent stem cells as the starting material for manufacturing. She says that ViaCyte is trying to overcome the limitations of islet transplants such as limited supply, high cost of organ procurement, inconsistent quality, and immunosuppression.

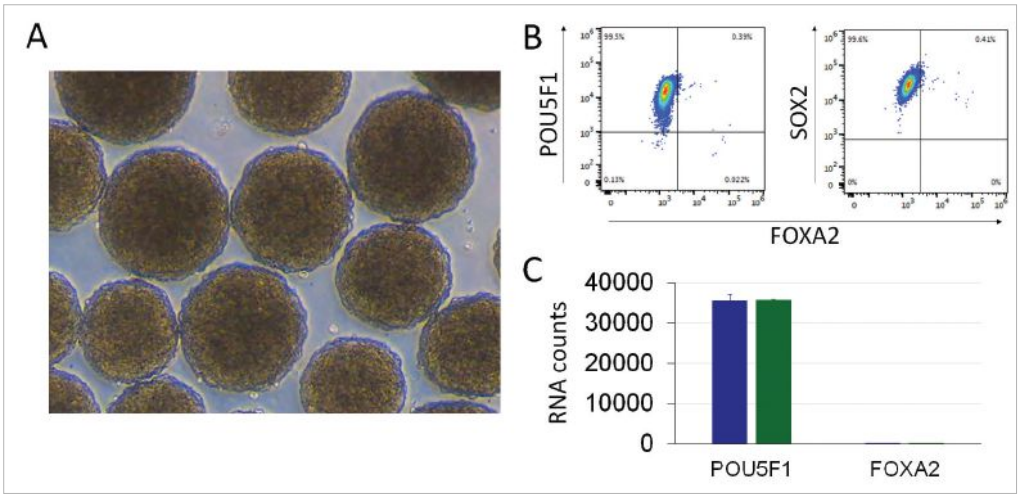
ViaCyte has a portfolio of three different product candidates that are based on its core technologies—human pluripotent stem cells, directed differentiation to pancreatic precursors, and a family of subcutaneous delivery devices. Its most advanced program, PEC-Direct, delivers pancreatic precursor cells in an open device that requires the use of immune suppression. PEC-Encap delivers the cells in a closed device designed to allow for nutrient exchange but protect the cells against immune rejection. A third preclinical program, PEC-QT, which ViaCyte is pursuing in partnership with **CRISPR Therapeutics**, aims to use gene editing to create cells that evade the immune system.

Kemp said that there is increasing demand for a supply of cells not just in the field of cell therapy but also in other fields, creating a need to manufacture a large amount of pluripotent stem cells, particularly as clinical development progresses. ViaCyte's initial production process used 2D flasks and 3D roller bottles.

"If we were to scale this out, basically replicate that process, the number of lots we would need to produce for the commercial phase becomes unrealistic very quickly," Kemp noted. "So, we need to develop a scaled-up manufacturing process to efficiently produce high-quality cells."

Scale-up hurdles include time and resource constraints, difficulties in starting the development process early enough, and the need to develop scale-up technologies that can meet stringent process and forecast requirements. Kemp highlighted the stirred-tank bioreactor as a scale-up technology with many advantages. For example, there are no surface area limitations, and processing may occur in a closed and controlled environment.

"We can consistently create aggregates of a specific size and then also expand them while maintaining pluripotency," she declared. "Similarly, with the differentiation process, we have



ViaCyte, a regenerative medicine company that develops novel cell replacement therapies, is working to grow human pluripotent stem cells (hPSCs) as aggregates in a 3D stirred-tank bioreactor to support scale-up production efforts. Culture conditions are first characterized at a small scale, and then optimal parameters are applied to larger scale bioreactors. (A) hPSCs after aggregation and expansion in a stirred-tank bioreactor. (B) Flow cytometry results show that the hPSC aggregates express proteins for pluripotency (SOX2 and POU5F1/OCT4) and not of proteins that would indicate spontaneous differentiation (FOXA2). (C) Similarly, gene expression analysis indicates comparable expression of genes for pluripotency (POU5F1/OCT4) when hPSCs are grown in 2D in flasks (blue) or as 3D aggregates in a stirred-tank bioreactor (green), and no expression of off-target genes (FOXA2).

Sebastian Rieck, PhD, principal scientist, ViaCyte

Cell Therapy Manufacturing Processes as Individual as the Products

a hundred runs under our belt where we've been able to demonstrate the ability to make pancreatic precursor cell aggregates efficiently and effectively in this 3D environment."

Playing catch-up

Manufacturing control in cell-based therapy products is sometimes limited due to a relative lack of product knowledge, suggested Mo Heidaran, vice president of technical, regulatory, and technical CMC consulting for cell, gene therapy, and tissue engineering at **Parexel International**. He noted that in some cases, manufacturing control for cell-based therapies may lag that for biologics by 15–20 years.

One of the biggest hurdles to overcome, he emphasized, is product consistency. "Many of these products have been developed in academic settings and are very complex," he said. "Characterization of the product is very challenging because of the biological complexity and poorly defined mechanism of action. One issue that has to be overcome is understanding the complex attributes of these types of products—and recognizing that the process really is the product."

With the addition of genetic modifications, including CAR T-cell therapies and edited cellular products, these technologies bring additional layers of complexity.

Considerations for manufacturing include how to deal with manufacturing changes, product quality assessment, challenges in collecting biological source material, compliance with donor eligibility requirements, donor-to-donor variability of starting materials, and limited shelf life of products. Establishing good manufacture control requires extensive characterization of the drug product by better understanding the relevant critical quality attributes, critical process parameters, and key process parameters.

Because the FDA has a phase-based approach for initiating Investigational New Drug Applications, there is more emphasis on the safety of products and less emphasis on control of manufacturing and consistency. As a result, companies tend to pay less attention to critical aspects of manufacturing control. Heidaran recommended that companies pay special attention to the development of appropriate phase-based manufacturing controls—especially if the companies receive expedited program designations and can proceed without having to introduce major manufacturing changes during a licensing trial. GEN

GEN ROUNDTABLE

Single-Cell Proteomics Tackles Vaccine Development

Vaccine development can be daunting. The overarching goal to create long-lasting immune protection requires an in-depth understanding of the immune system's response to an infectious agent, immune monitoring to determine and predict the building of the protective response, as well as cytokine-level monitoring for potential toxicities related to cytokine storms.

GEN spoke to five leading researchers to hear their views and strategies on infectious disease research and vaccine development.

***GEN:* How does understanding and detecting immune response and function affect developing therapies and vaccines for infectious diseases? How do single-cell tools play a role?**

Moriya Tsuji:

Before trying to develop vaccines or immunotherapies, we need to identify and characterize the immune response, particularly the protective response, against the infection. In human populations, a certain subset is susceptible and another resistant; the same is true in small animal models. It is important to compare the response of both populations at a single-cell level to understand what separates the two.

Moriya Tsuji, MD, PhD
Professor of Medicine, Aaron Diamond AIDS Research Center, Division of Infectious Diseases, Department of Medicine, Columbia University Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center

In small animal models, such as a mouse model, we can challenge both resistant and susceptible strains with a pathogen and monitor survival rates, as well as analyze single cells at different time points to determine which cytokines/chemokines dictate resistance or susceptibility. To determine the efficacy of vaccine candidates in vivo, animals can be immunized with each and challenged with the virus of interest. If one vaccine exerts more protective efficacy than others, you can then evaluate the differences in the immune response.

Using a cutting-edge, single-cell assay, you can also harvest specific tissues/organs from the vaccinated animals and measure the cytokines, T cells, B cells, macrophages, dendritic cells, etc. to see which cell types producing particular cytokines are most correlated with the protection. With this information, you can go back to the drawing board and design a vaccine that would induce such cell types and cytokine responses.

Jim Heath:

The immune system has heterogeneous cell types that work at different times in different roles. In a cascade of coordinated events, one expects that if something disrupts the process, the patient outcome is much poorer. The ability to monitor cellular activity over time to quantitatively map out an immune trajectory in an individual patient is key to understanding how similar patients are likely to respond and can inform on timing of therapeutic interventions. At one point you want your T cells to aggressively pursue the virus, but at another the inflammation can spiral out of control and you might want to suppress it.

In a normal, well-validated, double-blind clinical trial everyone begins at the same time, and, hopefully the ones who got the drug cross the finish line faster. In the COVID-19 pandemic, patients are randomly distributed around the track and you have no idea when some finish or if the drug had an effect. This confounds how to interpret patient response; you need detail.

If you just consider cytokines in the blood, you lose that whole concert of immune cell behavior, and you cannot interpret. Single-cell tools begin to parse that out. In this circumstance, you need to treat every individual patient as their own trial and you can only resolve that with single-cell tools.

Stanley Perlman:

For understanding immunizations, measuring antibody responses is very critical because you want to learn how people respond. The ultimate question is: if they are exposed to the pathogen in question again, will they get the disease? Single-cell sequencing and other measurements give you a fine-tuning of what is going on.

Single-Cell Proteomics Tackles Vaccine Development

Although they may not be the most critical thing in the beginning, if we have that information it can only help us in the end. In the beginning we just want to know what protection is and have we reached it with our vaccine or prior infections. Those are things we do not know yet with COVID-19.

Rong Fan:

Years ago, a review article by Mario Roederer demonstrated that using low-plex flow cytometry, you could show a handful of effector proteins/cytokines/chemokines produced by single T cells that determine the immune function. The ability to simultaneously produce different effector proteins correlated with the potency of immune cells against the infectious pathogens or the possibility to clear dysfunctional tissues, such as tumors. Single-plex T-cell activation assays remain the standard in the vaccine industry; however, they are not sufficient.

That led me to develop single-cell cytokine secretion profiling in a microfluidic device, which IsoPlexis commercialized; the primary purpose then was to use it to evaluate T-cell vaccines for AIDS, supported by Bill and Melinda Gates Foundation, which was the very first grant I received after joining Yale. With single-cell multiplex cytokine profiling tools, scientists are in a better position to develop the most efficacious treatments. For example, in COVID-19, some patients' T cells very likely become activated early on, overreact, and quickly go into a full life cycle and apoptosis. These patients exhibit a significant reduction in leukocyte counts. How to accurately measure the activation status of different immune cells upon exposure to the coronavirus is important to understand how to mitigate.

Tina Wang:

Immune response is important for vaccine development because if you can understand the immune factors, how they function and correlate with host protection, then you have a better idea of how to develop a vaccine. If you understand the immune factors involved in the pathogenesis, then you can use that as a target to develop a therapeutic approach for controlling active disease. Single-cell analysis can provide more information to understand immune cells on a single-cell basis and how the functional cells play a role in immune protection and pathogenesis. This helps to develop better vaccines and immunotherapies.

GEN: What models are available today to look at the innate and adaptive immune responses in relation to infectious diseases and cytokine storms?

Stanley Perlman:

We do not necessarily need other models; there are so many cases of COVID-19 that we can use human specimens. If we can use our human infections well, we can learn a lot about what is going on during infection. For COVID-19 there are several animal models where animals do not get very sick. They may not be totally useful because much of what we care about is people getting sick and having pneumonia, and immune responses that contribute to that pneumonia. For mild disease, you can look at monkeys, ferrets, hamsters, and mice but if for severe disease, animal models are not there yet.

Stanley Perlman, MD, PhD
Professor, Department of Microbiology and Immunology, Department of Pediatrics, University of Iowa

To have access to human samples, one needs informed consent and Institutional Review Board approval. Those are critical steps, but if one can use human samples, then you do not have a discussion about the relevancy of an animal model system.

Moriya Tsuji:

The innate and adaptive immune response against an infection can be measured in various animal models, including mice, rats, ferrets, hamsters, and nonhuman primates. All models have pros and cons. In nonhuman primates, there are ethical, financial, genetic background, and reagent issues. Ferrets have been used for influenza studies, and hamsters have been shown to be very receptive to COVID-19. The downside is that there are almost no immunological reagents available for these two species. In this regard, the mouse model has many advantages because there are many such reagents available. Furthermore, you can harvest organs easily to measure the tissue-specific innate immune response after infection or vaccination at early time points and the tissue-specific adaptive immune response at later time points, for example at 2, 6, and 12 weeks.

There is a big difference between the immune response of mice and humans. More than five years ago, I began generating a humanized mouse model that mimics the human immune system by transducing genes that encode human leukocyte antigens and human hematopoietic cytokines in highly immunodeficient mice, followed by the engraftment of human hematopoietic stem cells. In my work with malaria vaccines, this meant I could administer a human vaccine and measure a human immune response. Humanized mice are not a perfect model, since endothelial, epithelial, and other cells are still mouse-derived, and as a result, human viruses cannot infect well in challenge studies. We know that the coronavirus (SARS-CoV-2) infects humans via the angiotensin-converting enzyme 2 (ACE2),

particularly in lung alveolar cells. I am now designing a human immune system mouse which expresses human ACE2 in the lung to try and replicate human infections in a mouse model.

Tina Wang:

It is better to study the innate and adaptive response using in vivo models. Within vitro models, it is hard to understand unless you identify adaptive immune cells to study. In vivo you can study kinetically because the innate response is boosted very early and the adaptive immune response develops later. You can use tools, like specific antigens, to determine if the response is pathogen specific, which is related to the adaptive response. For vaccine development, the adaptive response plays a very important role because you have a memory response to help the host prepare and prevent disease.

It depends on the pathogen, but generally in preclinical studies we try to develop an animal model that can either partially or fully mimic the human disease so we can study immunity. This is the best way, but sometimes there is no feasible animal model—or there may be a model but a lack of critical reagents to study the immune response.

In those circumstances people also study in vitro, and in recent years organoid systems have become popular, which are a better way to mimic human disease than traditional cell culture. They provide a better system to understand infection and immune response. Groups used organoid systems to study the Zika virus.

When bacteria, viruses or parasites infect cells or animals, the immune system is boosted and you can measure innate cytokines. It has been reported in COVID-19 that a cytokine storm plays an important role in the viral pathogenesis. The main cytokines involved are the pro-inflammatory ones, such as IL-6, TNFα, and IL-1β.

Rong Fan:

In terms of the adaptive response, when T cells and B cells see antigen from the virus processed by antigen-presenting cells and presented on the surface, they recognize it, become activated, and then quickly expand, and the individual begins to adapt immunity against the pathogen. This happens annually in seasonal flu. If you already have a vaccine, antigen-specific T cells and B cells have been induced by the vaccination process and some turn into memory cells in circulation, then quickly respond when infection occurs.

Once activated, the cells are in a different state and produce different effector cytokines to battle the viruses themselves or viral-infected cells. Some return to memory stage and produce different proteins. At any given time of the infection, T cells or B cells are always a dynamic heterogeneous population undergoing a complex differentiation process and display a wide range of effector functions that is difficult to dissect. Looking at single cells in their full-range functional states and performing a highly multiplexed cytokine evaluation are very important to figure out what actually constitute such heterogeneous populations.

For the innate response, it is more complex. Scientific evidence shows that innate cells may have certain memory or can be trained to develop certain memory; although not so antigen specific, they can be trained to respond to certain infectious pathogens in a more effective and rapid manner, representing a new avenue to develop vaccines. To identify, characterize, and quantitate the cells involved and how they remember and respond to the different pathogens, you need a single-cell resolution and highly informative analytic tool.

Rong Fan, PhD
Associate Professor of Biomedical Engineering,
School of Engineering and Applied Science,
Yale University

Jim Heath:

You can harvest innate and adaptive immune cells from blood and look at their genetic regulatory network and surface markers. The adaptive immune response generally recognizes something very specific about the foreign entity through T-cell and B-cell receptors, which is why it takes time to develop that recognition. The genes for the receptor repertoire are built by genetic shuffling and are different for every T and B cell. This gives tremendous diversity from a small number of genes, but it makes sequencing and analysis difficult.

Using different assays, we can understand what specific fragments of the coronavirus (SARS-CoV-2) the immune cells are seeing. The coronavirus spike protein binds to human ACE2 receptors, part of it cleaves off and then another part flips around, and the virus injects the RNA through the cone. This protein is by and large most of what your acquired immune system is going to be able detect—the whole protein that B cells evolve to see or an antigen fragment that T cells see.

Some fragments will lead to protective responses, and some will just exhaust your immune system if the T cells are activated against fragments nonessential to the virus. Single-cell analysis of patient samples shows many exhausted T cells. At the heart of vaccine strategies is how to resolve what is protective versus immune dominant. The innate and adaptive models are the result of years of research. With single-cell technology, we

Single-Cell Proteomics Tackles Vaccine Development

can put these models to an extremely severe test at high resolution.

Cytokines are general molecules that help the immune system communicate. Cytokine storm is a symptom that many patients exhibit; if you can trace these cytokines, you can determine the source. It will not be the same for everyone, but it will have some common characteristics and is controllable. COVID-19 is complicated. We have the tools to parse through the complexity, but those tools are confounded by the very large heterogeneity in the patient population.

***GEN:** What are some challenges in preclinical vaccine and therapeutic development that can be overcome by having potency tools and better ways to characterize human immune response? Which cellular analysis tools will reveal this potent response?*

Stanley Perlman:

Generally preclinical means experimental animals. As a community, when we were doing these studies methodically and carefully, we wanted to make sure that whatever we used worked in small animal models, then worked in nonhuman primates and was safe without side effects. Typically, we would want to monitor experimental animals clinically and for immunopathology with blood samples or lung dissection, and we would conduct challenge studies and measure antibody and T-cell responses. Then we would move to people and do very methodical testing. A lot of that is circumvented or ignored by the urgency of the COVID-19 situation.

Now we are doing the same things, but we are doing them simultaneously to get vaccines up and running for human populations. Antivirals are different; there is no question that you have to show efficacy, and that has not always been done well. If you use a well-established vaccine platform and show safety, then you can move along a little faster. In other words, if we know that a particular vaccine platform works well, and then if we know it works well for a protein that is important for another human virus, and if all we did was use the same vaccine strategy but replace the known protein with a SARS-CoV-2 protein, there would be less worry that something bad would result.

When vaccines started in the 1950s, we knew little. We did the best we could and were lucky that the polio vaccine killed so well.

Tina Wang:

In order to better understand and characterize human immune response, we want to have a feasible animal model so we can study the infection in vivo to understand the immune response and also to determine whether the immune response plays an important role in protection or pathogenesis. For that reason, you want to know more functional detail since different cells may play different protective or pathogenic roles. You want to determine the multifunctional cells, and if you have a tool to do that it would assist a better understanding. Single-cell analysis allows you to understand the function on a single-cell basis, along with more dynamic features about the immune function.

The challenge for the preclinical study is a lack of tools to fully understand the immune cell function. If you have tools to understand, like microarray proteomics, you can find more information on single cells and the multifunctional aspects that can help you understand their role. This also depends on what pathogen you are studying. In general, the more potent the tool you have, the better the understanding of immune function.

Tian (Tina) Wang, PhD
Professor, Department of Microbiology & Immunology, Department of Pathology, Center for Biodefense and Emerging Infectious Disease, UTMB

You can also do experiments to determine how the immune system correlates with host function, for example, you can deplete the cells using antibodies, or use genetic marker or transgenic mice models to determine that particular immune cell function correlation with host cell protection or pathogenesis.

Moriya Tsuji:

One of the best methods available so far is IsoPlexis' multiplexed technology, which measures more than 40 cytokines/chemokines at the single-cell level. This is very powerful. You can pair this technology with other cell-based assays, such as multicolor flow cytometric analyses. Flow cytometry is well established and accessible, and with recent improvements you can determine dozens of surface markers on the same single lymphocyte.

In the case of COVID-19, sick patients exhibit lung pathology and secretion of IL-6, IL-10, TNFα, and possibly IL-1 in severe cases. These are most likely produced by lung alveolar macrophages, and the pro-inflammatory nature of these cytokines may be one of the causes of mortality. At the same time, the CD8+ and CD4+ T cells that secrete interferon gamma are reported to be depleted, most likely due to exhaustion. You need multiplexing tools to study these mechanisms in-depth.

Using multiplexed technology, you can determine the inflammation caused by the innate immune response. Knowing these details, you may be able to create a vaccine, for example, which elicits immune cells that would inhibit IL-6 in response to the infection in advance, and thus induces a protective

response to combat the pathogenic one in a preventative fashion.

Jim Heath:

If we know which immune responses are protective versus noisy, that can help us design a vaccine. Typically, multiple vaccines are developed simultaneously; there are a host of COVID-19 vaccines under development. Some are quite novel technologies. Now you can use the genetic material to make the neutered pathogen and not the neutered pathogen itself like the smallpox virus. This looks more like a natural course of infection and may possibly lead to a better immune response.

You can chop up the virus without the RNA, but we are not sure what is immunoprotective or noise. Coronaviruses change over time. If a vaccine worked against SARS-CoV-2 there is no guarantee that it will work in the future. If you really understood the details of the virus in terms of genetic mutations, you could design a vaccine that has a much longer protective mechanism. For COVID-19, the strategies and patient population are complicated.

Rong Fan:

The test of vaccine-induced antibodies in animals was often conducted by immunization of the animal against the viral component. Then, a simple binding assay allows us to see if the induced IgG antibodies can bind the viral proteins. To evaluate the vaccine-induced T cells, you can spike the viral peptide antigen in dendritic cells that present the antigen on the surface. A similar binding assay allows you to see whether the induced T cells can recognize the viral peptide antigen-specific signal.

But you do not know if the binding provides a functional consequence. It may occur but not induce the required immune responses to, for example, recruit other partner cells, mount antiviral activity, perform cytolytic function, and so on, in order to completely clear the infection; the T cells might just recognize the viral component but cannot do their job properly. Eventually you need an assay to confirm the functional efficacy. This is the most challenging and time-consuming step.

Characterizing the functional outcome allows determination of a signature that correlates with potency and durability to predict the most efficacious T- or B-cell responses and the corresponding vaccine candidate before you complete a six-month animal test. For example, using the coronavirus (SARS-CoV-2) spike protein, you immunize animals to develop the antigen-specific T cells. Then perform monoclonal expansions of the polyclonal population, and do detailed analysis of those T-cell functions. Next you sequence the T-cell or B-cell repertoire to determine which clone is the right therapeutic T cell. This is time consuming and should be carefully monitored at every single step as you cannot do many repetitions of the trial-and-error process given the urgency of this pandemic.

If single-cell functional signatures can predict the outcome in animal or patient, it would be faster to characterize the induced T cells without monoclonal expansion and then determine the functional profiles along with TCR sequencing to come up with the optimal design without a lengthy screening process to speed up the vaccine development. Single-cell tools give you that diverse characterization within several days.

GEN: What role do you think assessing and understanding a proteomic cytokine response from immune cells and more systematically in bulk will play in developing better vaccines and therapies for diseases such as COVID 19?

Moriya Tsuji:

I want to emphasize the importance of measuring the cytokine response. Recent data from China demonstrated that 10 out of 175 patients who recovered from COVID-19 were seronegative, which means that they had no antibodies. Nine out of ten of those seronegative patients were under 40 years old. This study suggests that other than antibodies, factors such as T cells and the cytokine response may have contributed to protect these seronegative patients.

In terms of the antibody (or humoral) response, many vaccines against the Dengue virus have been shown to produce antibodies that cause a phenomenon known as antibody-dependent enhancement (ADE). The induction of ADE increases virus infection, and as a result, these vaccines actually make the disease worse. This is why some scientists are hesitant to make a vaccine against COVID-19; there is some indication that some people may have antibodies that cause ADE. Therefore, you would have to design a vaccine very carefully in order to induce antibody response that mediates protection, but not ADE.

In view of this, multiplexed proteomic assessment of the cytokine response becomes quite important. The response could be from macrophages, T cells, or other cells, separate from the antibody response. This bulk and cutting-edge assessment could lead to the identification of an indicator of a protective or pathogenic immune response that could lead to morbidity and mortality. Such data may therefore have predictive value, and is particularly important for COVID-19 due to the urgent need for therapeutic or preventive vaccines.

Stanley Perlman:

Knowing more about the fine points of the immune response will help us make better vaccines. Until recently we did not

Single-Cell Proteomics Tackles Vaccine Development

have the potential to evaluate the cytokines/chemokines, etc., but now that really could help. You can measure single cells from people and measure the cytokines they make, do RNA analyses, flow cytometry—these tools are so sophisticated nowadays that on a single cell you can do fifty measurements, with RNA a thousand measurements. There are a lot of things we can do; a challenge is interpreting everything. We get a lot of data and you want to make sure you can make sense out of it.

Jim Heath:

The advantage of looking at immune cells at the single-cell level is that cytokines inform about function. You can tell the different roles of the immune cells and the different subroles of T cells by the functional cytokine signature. That is really important. The cytokines give you a real feeling of not only what the immune system is doing but also where it is headed near term. That is important for anticipating how the immune system is reacting to a vaccine or for diagnosing a cytokine storm.

If you take a drop of blood and look for certain types of T cells, like CD8+ T cells, you might have a million but probably about 1–10,000 are really dominating. If your T cells are going on the hunt against an infection, the queen bees controlling the hunt are the important ones to look at and understand. You can only see them if you do a single-cell analysis on a lot of cells to capture that small percentage; otherwise, it is very hard it is hard to resolve any of the hard questions.

James R. Heath, PhD
President and Professor, Institute for Systems Biology, Professor, Bioengineering, University of Washington and Department of Molecular and Medical Pharmacology, UCLA

Tina Wang:

COVID-19 is caused by a novel coronavirus that has a high homology with the SARS-CoV-1 virus but also a lot of difference in terms of virulence, transmission, and permissive cell type. A lot of things need to be known. A potent tool to provide more proteomic information would be very helpful to understand how immunity plays a role in the disease. We know in general that cytokine storm could be correlated with disease severity and ADE, and we need to understand the underlying mechanisms. A tool to analyze cytokines and other immune factors systematically would be helpful.

People are racing to work on vaccine development or immunotherapy. There are some antiviral and immunomodulatory candidates already, but almost none have been tried clinically. Using proteomic cytokine tools to analyze the data from patients who receive the trial therapies would be very helpful. Current reports are somewhat conflicting about the effects of antiviral agents because some were being used very urgently. There was no clinical trial specific for COVID-19; very limited data from COVID-19 patients are available.

If there is ADE that information is helpful to determine the design of the vaccine. Currently scientists are using different approaches for vaccine development until there is an effective one. There are many vaccines, such as inactivated virus, RNA, DNA, or recombinant proteins, and they induce different types of immune response. In vivo studies in animal models will test if ADE is induced during challenge studies.

In vaccine development, the minimum time is four or six months for the preclinical and clinical stages but could be much longer, especially if you want it for a particular strain of SARS-CoV-2. SARS-CoV-1 virus has already shown the ADE phenomenon, so you have to be careful to assess safety. Even if it is okay in an animal model, you still have to be careful when you go to clinical trial.

Rong Fan:

Functional characterization of single immune cells is very important to better help you identify the top candidates during vaccine development. Eventually you need to look at the B cells and the antibodies circulating in blood, and whether there are enough of those and which cytokines are presented in blood to understand the systemic response. If there are adverse effects, you need to monitor what is going on in preclinical development as well as during clinical trials.

My research includes profiling of blood from COVID-19 patients, and we have seen many cytokines that are critical to monitor, especially in the lung. The early and later stages of infection are different opportunities. In the early stage during viral expansion, you can control and suppress expansion.

In the later stages, the virus is not the problem rather a systemic cytokine storm and organ failure. Antiviral therapies would no longer help. We need to determine how to analyze the system pathology in those patients and specifically tackle the problem by suppressing pro-inflammatory response. The response appears to be different across multiple patients and calls for precision medicine. ■

From the Cultural Revolution to the Gene Therapy Revolution

An Interview with Guangping Gao, PhD, a Pioneer of Viral Vector Gene Therapy for Rare Genetic Diseases

Guangping Gao, PhD, is professor and director of the Horae Gene Therapy Center at the University of Massachusetts Medical School in Worcester, MA. Over the course of three decades, Gao has made profound contributions in the area of adeno-associated virus research, initially working with James M. Wilson, MD, PhD, director of the gene therapy program at the University of Pennsylvania. Gao has received multiple honors in recognition of his service, expertise, and dedication. For example, he was named president (2019–2020) of the American Society of Gene and Cell Therapy.

Gao has published 250+ research papers, six book chapters, and four edited books, and has fulfilled editorial responsibilities for several gene therapy and virology journals, including the *Human Gene Therapy*, a journal that Gao currently serves as deputy editor-in-chief. Gao recently spoke to Kevin Davies, PhD, executive editor of *Human Gene Therapy*, about his remarkable life journey and hopes for the future of gene therapy. (The interview originally appeared in *Human Gene Therapy*, Vol. 31, Nos. 3 and 4, DOI: 10.1089/hum.2020.29109.int, published by Mary Ann Liebert. Kevin Davies, PhD, executive editor of Human Gene Therapy, conducted the interview, which has been lightly edited for length and clarity.)

We will get to your preeminent research and leadership in the gene therapy field, but let's start at the beginning.

Gao: I grew up in China during the Cultural Revolution. Around 1975, I was compelled to leave my studies and go to the countryside to receive additional "education" from farmers and peasants. My dream about new medicine really starts there. I interacted with farm laborers on a daily basis, and I saw many of them suffer from various diseases and painful conditions.

I was trying my best to use acupuncture and traditional medicine to help them, but I wished I could have some "magic medicine" to make a more substantial impact, particularly for the elderly and people with cancer.

In 1978, I was one of the first generation of students to enter college after the Cultural Revolution. I was admitted to a medical university in Chengdu, Sichuan. I worked on drug development and medicinal chemistry. In 1988, I graduated from the university and got an opportunity to come to the United States, sponsored by the World Health Organization (WHO). I was looking for opportunities to develop the next generation of medicines that I had dreamed about back on the collective farm.

I started my PhD at Miami Children's Hospital and Florida International University with my mentor, Reuben Matalon, a pediatrician and medical geneticist. He was a prominent researcher on rare diseases such as Tay-Sachs, Hurler, and Gaucher. His major contribution as a geneticist was the discovery of the biochemical defect in an inherited leukodystrophy called Canavan disease.

I remember it well—I published that paper in the early days of Nature Genetics!

Gao: Yes, thank you! I joined his lab in 1989. My assignment was to isolate the genes and the mutations responsible for Canavan disease. Working with my lab mentor, Rajinder Kaul, I discovered the gene and mutations for Canavan disease and published my thesis

Guangping Gao, PhD, Professor and Director, Horae Gene Therapy Center, University of Massachusetts Medical School

From the Cultural Revolution to the Gene Therapy Revolution

work in *Nature Genetics* in 1993.[1]

After that, I asked myself, what's my next step? Because we saw many Canavan patients at these centers, we knew exactly what was going wrong with those kids. We had to figure out a way to fix it. In 1993, I decided to look for the next generation of medicine, specifically at the opportunities in gene therapy for genetic disorders. Finally, Jim Wilson accepted me as a postdoctoral fellow at the University of Pennsylvania's Institute for Human Gene Therapy.

The first task Jim gave me was to create new generations of adenovirus. At that time, adenovirus vector was much hyped because it has a high transduction efficiency. Because we knew adaptive immunity/immunotoxicity is a major issue for adenovirus, we decided to cripple the virus further to make it more replication defective. This might prolong transduction efficiency and stability in tissues.

I spent about two years there, first making a cell line to complement the crippled virus. Then we used that cell line to create the further-crippled virus. (You need to transcomplement its growth with E1 and E4.) They called this third-generation virus at the time. We demonstrated that, yes, virus can reduce liver toxicity in mice and immunotoxicity and prolong expression substantially.

When I published that work in 1996,[2] I said to Jim, "I'd like to move on and start my career in industry because I have two kids to raise." I was 38 at the time. He said, "No! Why leave? I'm going to give you a job." He told me they were trying to apply the next-generation adenovirus vector for clinical trials. There was a lab called the Human Applications Lab, a GMP facility at Pennsylvania Hospital where scientists were trying to grow the virus for multiple clinical trials, but they could not grow it well.

My career in gene therapy started from there. I spent about two years making the virus work. In the first two weeks, I was able to generate high quantities of virus. Jim was in his office, talking to a reporter from the *Philadelphia Inquirer*. I told Jim, "I got the virus, and they are 10^{13} or 10^{14}." Jim said to the reporter, "Now we can even swim in this gene therapy vector!"

By that time, we were doing several clinical trials in cystic fibrosis, ornithine transcarbamylase (OTC), mesothelioma, and others. By early 1998, we wanted to look for new viruses,

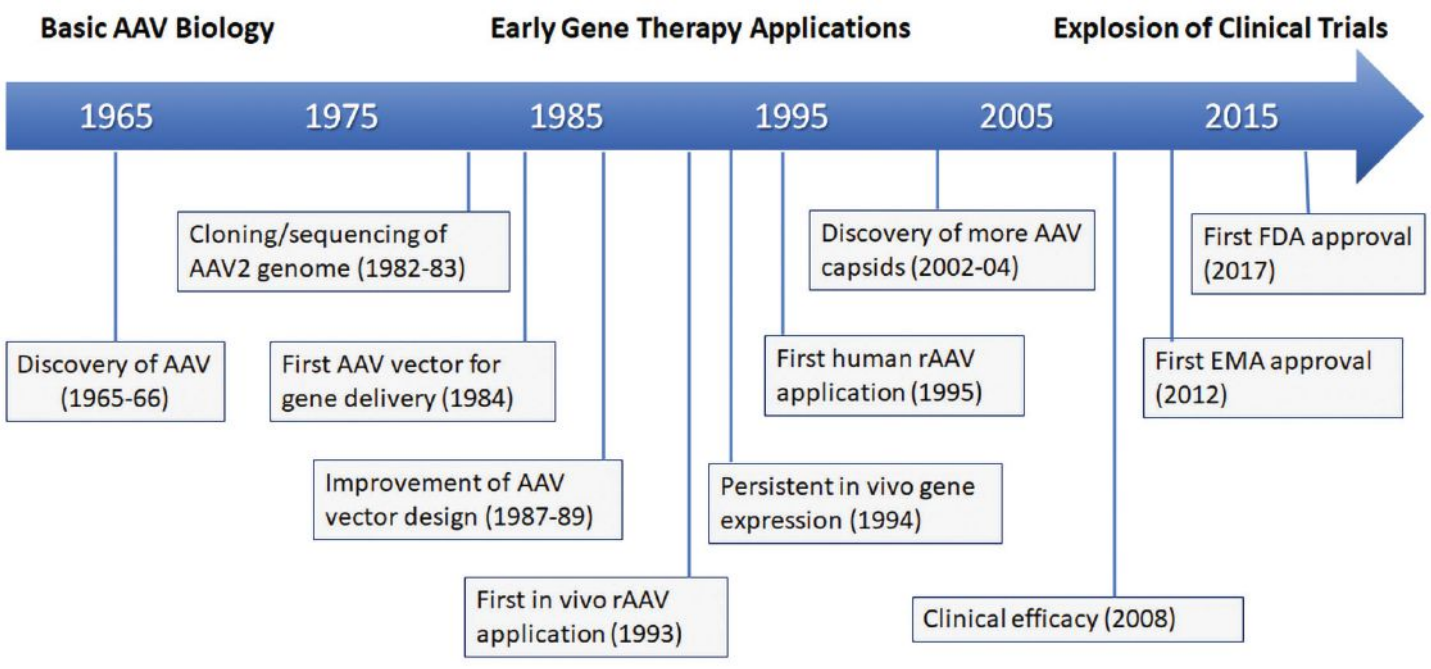


Figure 1. Timeline of major events in adeno-associated virus discovery, characterization, and clinical deployment.
Adapted from Wang *et al.*[7]

the next generation of gene delivery vehicles. I started working with AAV prototypes such as AAV-2, AAV-1, and AAV-5. Those were the first serotypes to attract a lot of interest and development.

Who first identified AAV? Was it discovered serendipitously?

Gao: Yes, it was discovered in 1965 from some adenovirus preps. They called it adeno-associated virus (AAV) because when they purified the adenovirus and looked at it under a microscope, it was a very small virus in the company of the much larger adenovirus.[3]

I think Arun Srivastava and others sequenced AAV. Nick Muzyczka, Jude Samulski, Barrie Carter, and others started vectorizing—demonstrating you can create a vector in transduced cells very easily. Many groups then demonstrated that AAV can transduce animals in vivo. The difference is that adenovirus only sustains for a maximum of two to four weeks. But AAV—at that time, primarily AAV-2—can sustain for hundreds of days.

My first task with Jim was to figure out how to produce a scalable manufacturing process. I started making cell lines, creating adeno-AAV hybrids. I published a paper in 1998.[4] We converted a transfection-infection system into a total infection system that generates tons of AAV. Working with my colleague Guang Qu, we developed a column purification system using heparin-binding columns in early 2000.

Then on September 17, 1999, this tragic event with adenovirus OTC gene therapy happened, and we lost 19-year-old Jesse Gelsinger. For the entire field, it was a drop from a peak to a deep valley. We experienced 10 years of dark ages for gene therapy.

I continued my AAV work. We started the first AAV-2 limb-girdle dystrophy clinical trial with Jerry Mendell (Nationwide Children's Hospital, Columbus, OH) and colleagues at Penn such as Hansel Stedman and Lee Sweeney. We started the trial using the vector produced with my manufacturing methods under GMP conditions.

After the Gelsinger tragedy, was there added urgency and commitment to establish AAV as an alternative vector?

Gao: Absolutely. We started working with adenovirus, based on the discovery by Yiping Yang (formerly at Duke, now at Ohio State). He discovered immunotoxicity of adenovirus. My job was to reduce that adaptive immunity to adenovirus. But we overlooked this innate immunity, this cytokine storm, which killed Gelsinger.

> Generating large quantities of highly potent virus is the number-one barrier we face in the field.

I had initially started with AAV-2, but we did not really think about AAV-1 and AAV-5, or about discovering new AAVs, until Gelsinger. Then we realized, when you compare the two vectors, adeno is much more efficient. But for immunotoxicity, AAV is much, much better than adeno. Jim and I thought, if we can find a virus as efficient as adeno but without immunotoxicity, that should be the future of gene therapy. Gelsinger was an additional driving force for me to discover new AAVs.

I started work in 2001, and soon we discovered a library of new AAVs in nonhuman primates. We published our first paper in 2002.[5] That paper became the hottest paper in the field and gave us new hope to work on the next generation of gene therapy vectors.

How did that discovery come about?

Gao: Back in the winter of 2001, after we found some virus sequences, I presented the PCR data to Jim Wilson at a lab meeting. I could tell his mind was spinning: "Is this real or not?" After the meeting, he said, "Guangping, I think you stepped on a goldmine."

I started with nonhuman primates. We found that we can detect AAV in any animal. You never run into anyone with absolutely no AAV. It is in any tissue. In any PCR reaction, I always found multiple AAVs. That tells you how diverse [it is], how rapidly AAV is evolving. Then we published our second paper about nonhuman primate viruses, demonstrating AAV evolution.[6]

At what point did you expand or focus the search for new AAVs in humans?

Gao: You can find AAV everywhere. You can find a different AAV in the same samples. That's why AAV is amazing to me! As the initial discovery was based on nonhuman primates, I asked Jim in late 2002, "Should we move into human tissues?" He agreed. We discovered AAV-9, which is the first "super virus" for gene therapy from humans, in January 2003.[7] Our objective was to develop AAV to be as potent, as efficient, as adenovirus for transduction. But we wanted them to have much less immunogenicity. I think we accomplished that (*Figure 1*).[8]

We did not go through the traditional viral isolate characterization. We focused on PCR amplification of the capsid because we realized biology is only determined by the capsid. We didn't need anything else. We designed PCR primers in the conserved region and amplified through hypervariable regions, generating a new virus capsid with new biology.

To read the remainder of the article and see the references go to GENengnews.com and click the magazine tab.

DEVELOPING THE NEXT GENE THERAPY IS SCIENCE. MANUFACTURING REAL CURES IS ART.

Successful gene therapies are built on the combination of scientific advancement in DNA technologies and the art of manufacturing real cures.

Catalent Gene Therapy combines the latest viral vector technologies and proven experience in process and analytical development with the art of creating commercial-ready manufacturing processes to expedite your therapeutic to the clinic for waiting patients. **Catalent Biologics, where science meets art.**

Catalent
CELL & GENE THERAPY

us + 1 888 SOLUTION (765-8846) **EUR** 00800 8855 6178 biologics.catalent.com/gene