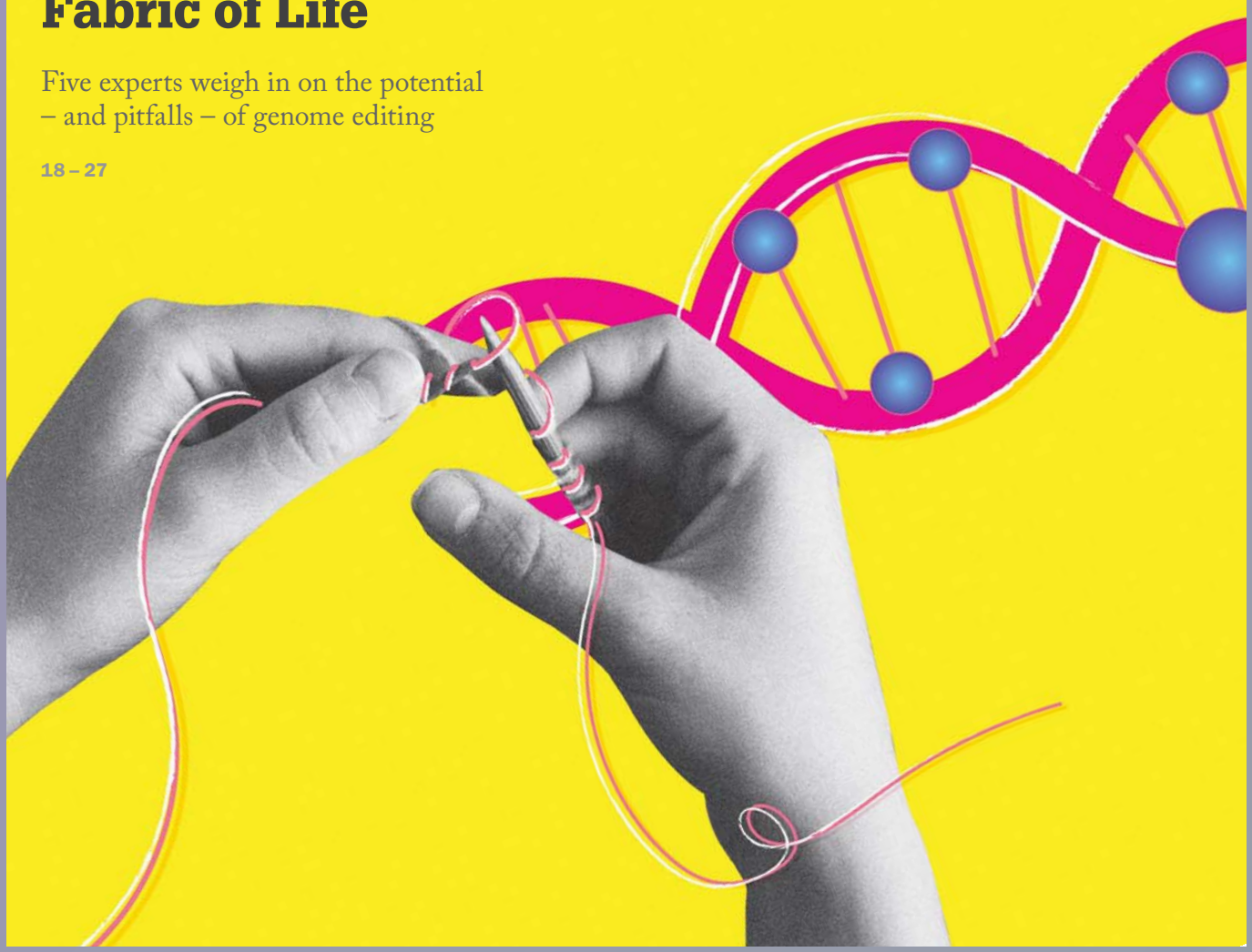
MAY / JUNE 2023 # 91

the Medicine Maker®

Reknitting the Fabric of Life

Five experts weigh in on the potential – and pitfalls – of genome editing



RESILIENCE

HOW TO

PULL YOUR FINISH LINE CLOSER.

It's easier than you might think:

- First, collaborate with a full-service biomanufacturing organization that's willing to explore aligned incentives and partnership programs that give you ultimate flexibility.
- Second, choose a partner that's investing heavily in drug product innovation to optimize every component of fill/finish.
- Your final step? Celebrate how easy it was to achieve the first two steps through a single call to Resilience.

Our goal is your goal: To shorten your timeline, and get your innovation to more patients, sooner. Let's start today.

Resilience.com

Your Reputation Precedes You, Pharma

Is the industry's poor reputation a factor in the Amgen–Horizon debacle?

We have all noticed discussions on drug pricing heating up in recent years – particularly in the US – but the impact on pharma company acquisitions was perhaps unanticipated. The Federal Trade Commission (FTC) has filed a bold lawsuit to block Amgen's acquisition of Horizon Therapeutics – because of drug pricing and, ultimately, strong mistrust in the pharma industry. A number of US states have also joined the lawsuit.

Two of Horizon's drugs – Tepezza and Krystexxa – currently have no competition on the market. And the FTC is concerned that the acquisition would "enable Amgen to use rebates on its existing blockbuster drugs to pressure insurance companies and pharmacy benefit managers (PBMs) into favoring Horizon's two monopoly products."

FTC's Bureau of Competition Director Holly Vedova delivered a damning commentary on the industry: "Rampant consolidation in the pharmaceutical industry has given powerful companies a pass to exorbitantly hike prescription drug prices, deny patients access to more affordable generics, and hamstring innovation in life-saving markets. Today's action – the FTC's first challenge to a pharmaceutical merger in recent memory – sends a clear signal to the market: The FTC won't hesitate to challenge mergers that enable pharmaceutical conglomerates to entrench their monopolies at the expense of consumers and fair competition."

For the lawsuit to succeed, however, the FTC will need to provide evidence that its concerns are merited. In its own statement, Amgen says, "The FTC's claim that Amgen might "bundle" these medicines (offer a multi-product discount) at some point in the future is entirely speculative and does not reflect the real world competitive dynamics behind providing rare-disease medicines to patients. And we committed that we would not bundle the Horizon products raised as issues; however, the Commission still decided to pursue this path."

If the FTC distrusts the industry so much that it is blocking deals, perhaps it's the wakeup call pharma needs to address its poor reputation. The use of the words "first challenge" in the FTC statement is ominous; it seems highly likely that the FTC will be looking at deals very closely in the future.

What are your thoughts? Are you surprised by the FTC's move? And what other actions may we see as a result of continuing discussions around drug pricing?

Stephanie Sutton
Editor

Contents

07

34

08

On The Cover

How genome editing is opening up the door to new therapeutics

Feature

Core Topics

NextGen

Best Practice

Sitting Down With

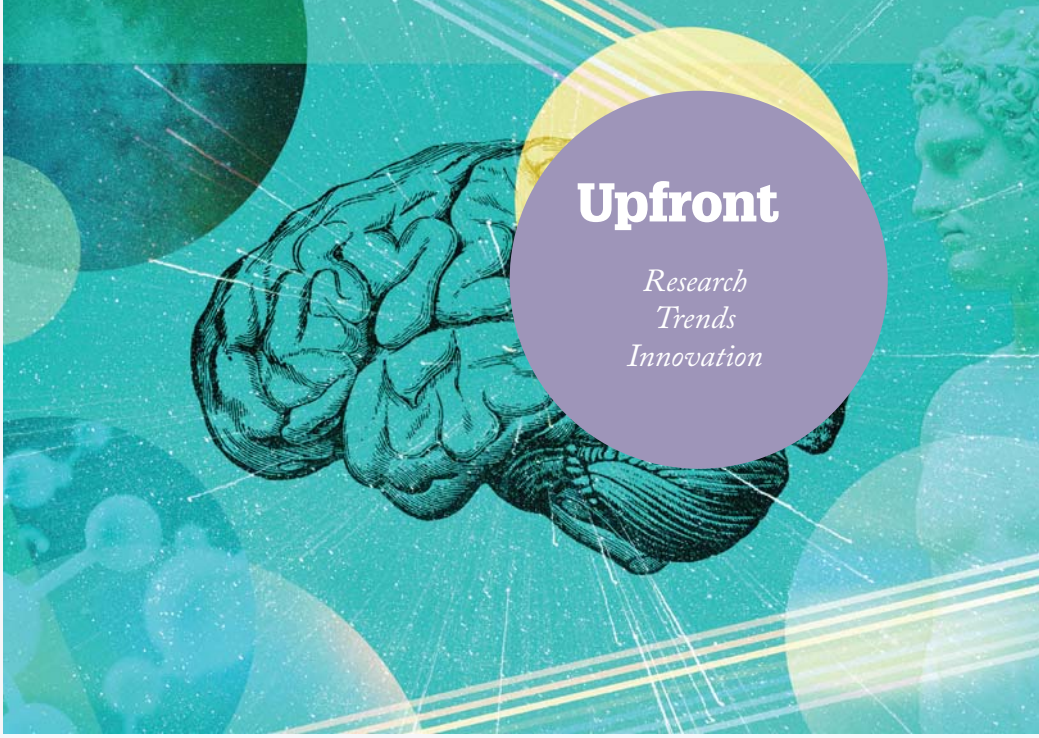
the Medicine Maker

ISSUE 91 - MAY / JUNE 2023

Change of address info@themedicinemaker.com
Tracey Nicholls, The Medicine Maker, Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK

General enquiries
www.texerepublishing.com | info@themedicinemaker.com
+44 (0) 1565 745 200 | sales@themedicinemaker.com

Distribution: The Medicine Maker (ISSN 2055-8201), is published bi-monthly by Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK. Single copy sales £15 (plus postage, cost available on request info@themedicinemaker.com). Non-qualified annual subscription cost is £110 plus postage



Anti-Anxiety

Researchers identify a brain pathway that holds promise for future anxiolytic drugs

A UK-led team of researchers has discovered a new amygdala miR483-5p/Pgap2 pathway through which the brain regulates its response to stress. The study focused on groups of microRNA molecules in animal models that regulate gene expression and target proteins that control cellular processes in the amygdala (1). The same group of molecules are also found in the human brain.

Following acute stress, the team analyzed the expression patterns of microRNAs in the amygdala of mice, and observed an increase in miR483-5p – a molecule responsible for suppressing expression of a gene called Pgap2. Pgap2 drives changes in the neuronal morphology of the brain and in behavior associated with anxiety. Together, the researchers showed that miR-483-5p acts as a molecular brake that offsets stress-induced amygdala changes to promote anxiety relief. "Remarkably, the mice with decreased Pgap2 quickly learned that the stress they were exposed to – although unpleasant – was not a direct threat, allowing them to cope better. As a result, they did not develop anxiety, whereas mice with normal levels of Pgap2 did," said co-author and Principal Molecular Biologist at Bitrobius Genetics, Mariusz Mucha.

According to the research team, around a quarter of us will be diagnosed with an anxiety disorder at least once in our lifetimes. The efficacy of current anxiolytic drugs is low; more than half of patients do not achieve remission following treatment. Limited success is perhaps linked to a lack of understanding on the neural circuits and molecular events that underly anxiety and stress-related neuropsychiatric states.

Valentina Mosienko, lead author and an MRC Fellow and Lecturer in Neuroscience in Bristol's School of Physiology, Pharmacology and Neuroscience, adds, "Stress can trigger the onset of a number of neuropsychiatric conditions that have their roots in an adverse combination of genetic and environmental factors. While low levels of stress are counterbalanced by the natural capacity of the brain to adjust, severe or prolonged traumatic experiences can overcome the protective mechanisms of stress resilience, leading to the development of pathological conditions, such as depression or anxiety."

The researchers now aim to explore various strategies that can modulate anxiety levels by manipulating the expression of these molecules.

Reference

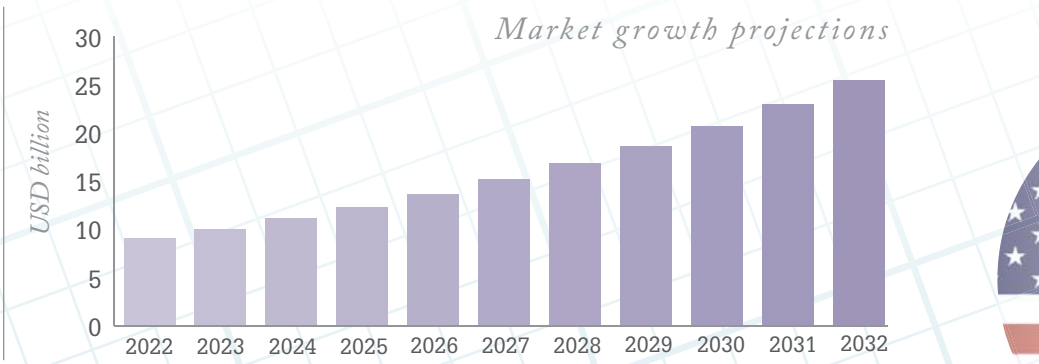
1. *M Mucha et al., "miR-483-5p offsets functional and behavioural effects of stress in male mice through synapse-targeted repression of Pgap2 in the basolateral amygdala," Nature Communications, 14, 2134 (2023). DOI: 10.1038/s41467-023-37688-2*

INFOGRAPHIC

The ADC Outlook

What do reports forecast for the ADC market? It's looking very positive.

Source: Precedence Research, Antibody Drug Conjugates Market (2023). Available at: www.precedenceresearch.com



US DRUG PRICING LAWSUITS - IN-BRIEF

What's going on with drug pricing lawsuits in the US? Here are four key points to bring you up to speed.

- Merck Sharp & Dohme is suing the US government over the Inflation Reduction Act's drug negotiation program, claiming it violates the First and Fifth Amendments. The lawsuit describes the negotiation program as "sham" and "tantamount to extortion."
- US politicians have hit back, accusing MSD of prioritizing profits over patients. Various experts believe that MSD may face an uphill battle in court. Public Citizen described the lawsuit as "desperate" and David Mitchell from Patients for Affordable Drugs said, "Want to know how frivolous the Merck anti-Medicare negotiation lawsuit claims are? One expert put it this way: There are 'better odds that Elizabeth Holmes wins Medtech Innovator of the Year than that Merck wins its lawsuit.'"
- Some MSD shareholders, who are members of the Interfaith Center on Corporate Responsibility, have released a statement denouncing the lawsuit. The statement says, "If Merck truly puts its patients and society first, then the company should align its statements with its actions. This action suggests that Merck is willing to protect profits even if it comes at the expense of patients. Needless to say, the inappropriate use of corporate resources and misuse of the U.S. legal system to file this lawsuit against HHS is not what we would expect from a company espousing the values of greater access and affordability."
- The US Chamber of Commerce is also launching a lawsuit over drug prices. As with MSD, the lawsuit focuses on the drug price negotiation program. The lawsuit says: "Congress created an unprecedented, one-sided regime that forces manufacturers to sell drugs at government-set prices. The appropriate term for this is 'mandated price control,' not 'negotiation.'"

Solid Tumor Success

Astrazeneca releases promising findings from ongoing ADC trial for solid tumors

AstraZeneca has released positive results from an interim analysis of the ongoing DESTINY-PanTumor02 phase II trial for Enhertu – a specifically engineered HER2-directed ADC. The analysis found clinically meaningful and durable responses across a broad range of HER2-expressing advanced solid tumors in previously treated patients. Specifically, participants showed a confirmed objective response rate (ORR) of 37.1 percent; a greater response was observed in patients with the highest level of HER2 expression, where the confirmed ORR hit 61.3 percent. Cristian Massacesi, Chief Medical Officer and Oncology Chief Development Officer, AstraZeneca, said: "Enhertu is the first treatment to demonstrate broad activity across HER2-expressing solid tumors where there are currently no approved HER2-directed therapies. This data will support our ongoing conversations with global health authorities as we look to bring Enhertu to as many patients as possible."

Key facts

- **North America region dominates the market**
- Breast cancer and blood cancer segments are areas to watch
- **Cleavable linker segment holds the most notable market share in terms of technology**

Noteworthy players

- **Takeda**
- Pfizer
- **GSK**
- AstraZeneca
- **Seagen**
- ADC Therapeutics
- **Gilead**
- Roche

Latest approval

- **FDA approves Genentech's Polivy in combination with Rituxan, cyclophosphamide, doxorubicin and prednisone**

Lonely Hearts Club

Researchers in Germany create an organoid that emulates the development of the human heart

Until now, the mechanisms underlying early epicardial development in humans have remained largely unknown. The human heart forms approximately three weeks after conception, making it almost impossible to study its development in the native context. Though scientists have long used animal models (mostly rodents) to investigate the mechanisms of heart development and function, results from such studies are not always translatable to humans due to the differences in organ size, physiology, and gene expression.

This led researchers from the Technical University of Munich (TUM) to develop self-organizing human pluripotent stem cell-derived epicardioids – otherwise known as organoids (1). Pluripotent stem cells were manipulated to form a "mini-heart" structure, comprising approximately 35,000 cells. Over several weeks, the cells were exposed to specific signaling molecules that mimic the natural pathways regulating heart development.

The resulting organoids, about 0.5 mm in diameter, displayed remarkable functionality. Despite lacking blood pumping capabilities, they could contract and respond to electrical stimulation. In fact, these organoids were the first to combine heart muscle cells (cardiomyocytes) with cells from the outer heart wall (epicardium). Previous models only incorporated cardiomyocytes and cells from the inner heart wall (endocardium).

The study also shows that early epicardioids contain a population corresponding to so-called juxta-cardiac field cells, which were recently discovered in mouse embryos as a common progenitor pool of the myocardium and epicardium. These precursor cells give rise to the epicardium and may exist transiently in the human body. Understanding their dynamics could offer insights into the regenerative potential observed in fetal hearts and potentially contribute to novel treatments for heart conditions, including heart attacks.

"Our new epicardioid model is the first to show the morphological and functional self-organization of heart muscle (myocardium) and the outer layer of the heart (epicardium)," says Alessandra Moretti, co-author and professor of regenerative medicine in cardiovascular disease at TUM. "The epicardium plays key roles in heart development and regeneration, and this model can therefore offer unprecedented possibilities to study human heart development, function, and disease in vitro."

Reference

1. *AB Meier et al., "Epicardioid single-cell genomics uncovers principles of human epicardium biology in heart development and disease," Nature Biotechnology (2023). DOI: 10.1038/s41587-023-01718-7*

Superbugs Versus Supercomputers

Antivirulence treatments could curb the threat of antimicrobial resistance

Antimicrobial resistance-related infections account for over 700,000 annual deaths, but are projected by the WHO to rise to as many as 10 million by 2050. To aid the development of new and effective treatments for drug-resistant bacterial infections, researchers from Simon Fraser University, Canada, have identified pathogen-associated genes in various disease-causing bacteria that could lead to new antivirulence drugs.

Using computational analysis and the university's "Big Data Hub" to examine thousands of previously sequenced bacterial genomes, the research team identified "antivirulence" drug targets that can disarm bacteria without causing resistance to develop. Antivirulence therapies do not "inhibit bacterial growth in vitro, but limit the production or function of virulence factors that promote infection or incite host damage in vivo" (1).

Reference

1. *Ford Caleb A., et al., "Antivirulence Strategies for the Treatment of Staphylococcus aureus Infections: A Mini Review" Frontiers in Microbiology (2021) 11. DOI:10.3389/fmicb.2020.632706*

SK pharmteco

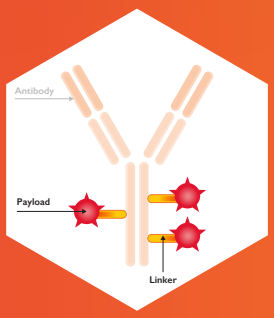
Small Molecule & C&GT

Your partner in...

Continuous Processing

HPAPI

Linkers & Payloads



Analytical Services

Chromatography Batch & SMB

Particle Engineering

Spray Drying & Micronization

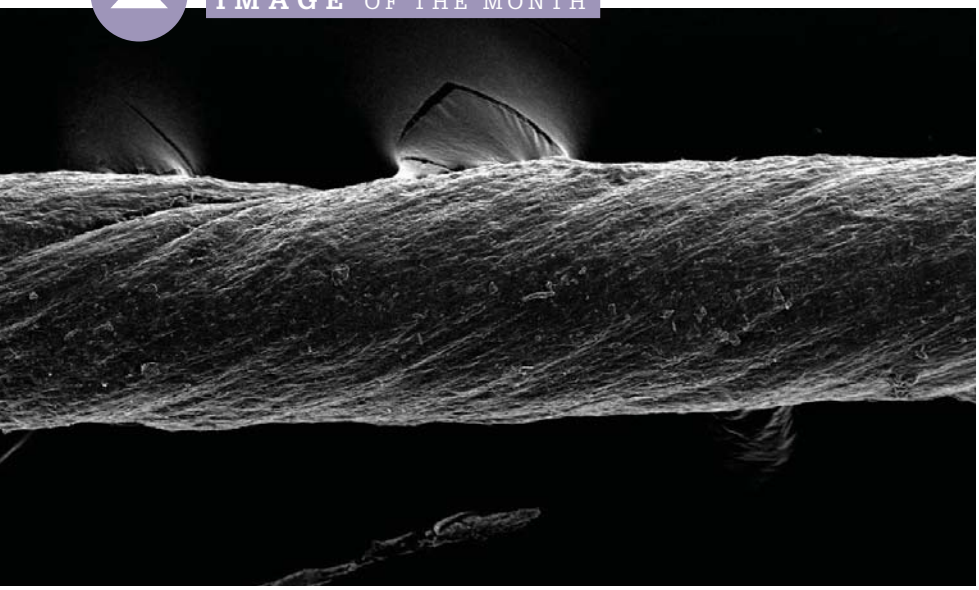
Cell & Gene Therapy

US | Korea | Ireland | France

skpharmteco@sk.com

www.skpharmteco.com

IMAGE OF THE MONTH



Back to the Suture

Inspired by sutures developed thousands of years ago, MIT engineers have designed "smart" sutures derived from animal tissue coated with hydrogels that can be embedded with sensors, drugs, or even cells that release therapeutic molecules. Credit: MIT

Would you like your photo featured in Image of the Month?
Send it to rob.coker@texerepublishing.com

QUOTE of the month

"The #COVID19 Emergency Committee met for the 15th time and recommended to me that I declare an end to the public health emergency of international concern. I have accepted that advice. With great hope I declare COVID-19 over as a global health emergency."

Three years after COVID-19 brought the world to a standstill, the World Health Organization has announced that it is no longer a global health emergency.

Credit: @DrTedros (Tedros Adhanom Ghebreyesusm director-general of WHO).

Nut a Problem

New immunotherapy may desensitize peanut-allergic toddlers

A growing body of literature suggests that consuming peanuts during infancy may significantly reduce the risk of developing an allergy in later life. Scientists have conceived of numerous approaches to desensitize the body to allergens (such as peanuts), but there are currently no FDA-approved options for children under the age of four years. That soon may change with DBV Technologies' epicutaneous immunotherapy (EPIT) – the aptly named Viaskin Peanut. Phase III trial results showed that Viaskin Peanut was statistically superior to placebo in desensitizing toddlers under the age of four years to peanuts after 12 months of treatment. The therapy, which is delivered using a patch, harnesses the immune properties of the skin to modify underlying food allergies and has the potential to desensitize the immune system to other allergens. The trial's primary efficacy endpoint test was met – and the results aligned with the safety profile observed in prior clinical trials involving children aged four years and older with peanut allergies.

BIOSYNTH®

Securing Life Science Supply Chains

Small Molecule Manufacturing Services

- **Custom Synthesis**
- **Large Scale Manufacture**
- **GMP Manufacture**
- **CDMO Services**
- **Experts in Complex Chemicals**

Our end-to-end manufacturing services take you from the first idea to the finished product. Scale and Quality to secure your supply chain with ISO and GMP capability.

www.biosynth.com **sales@biosynth.com**

In My View

Experts from across the world share a single strongly held opinion or key idea.

Putting Real-World Data to Work

As the FDA looks to further integrate real-world data and evidence into clinical research and approval processes, only high-integrity data will do

By Karen Ooms, Joint Chief Operating Officer of Statistics at Quanticate

Companies and regulators are looking for ways to support approvals of new indications for drugs already approved under existing indications and to satisfy post-approval study requirements. To this end, real-world data (RWD) and evidence (RWE) are growing in importance. Helping to provide clear insights on the status, role, and requirements of non-interventional studies, these forms of information could help transform the way study design and the approval process work.

The FDA has already drafted guidance on integrating RWD and RWE into clinical research, product approvals, and post-approval monitoring of drugs with the aim of clarifying expectations of these forms of data in pharma operations. But, in my view, it's important to define these data types and have tangible examples of how they could benefit us as drug developers.

For the purposes of its newly drafted framework, FDA defines RWD, and RWE as follows: "Real-world data are data relating to patient health status and/or the delivery of health care routinely collected from a variety of sources. Real-world evidence is the clinical evidence about the usage and potential benefits or risks of a medical product derived from an analysis of RWD."

With respect to clinical trials, the FDA notes explicitly that it is important to distinguish between the trial designs and studies that will be covered by the RWE program. Under the FDA's program, evidence from traditional clinical trials will not be considered RWE. However, hybrid or pragmatic trial designs and observational studies could generate RWE. The FDA's RWE program will cover clinical trials that generate RWE in some capacity (that is, sources other than traditional trials) and observational studies.

The guidance covers a variety of topics ranging from the role and requirements of non-interventional study designs to approvals of new indications for drugs already approved under existing indications. One of the key considerations regulators want to highlight is the quality and integrity of RWD it seeks to obtain from developers, manufacturers, and dispensers of pharmaceuticals.

The FDA also acknowledges that the increased use of electronic data systems in the healthcare setting has the potential to generate substantial amounts of RWD. According to a recent Deloitte survey on the pace of digitalization, biopharma companies have traditionally been slow to adopt digital technologies including AI, cloud, and the Internet of Things (IoT) in their operations. However, the analysts also found that certain digital technologies, such as the cloud (49 percent), AI (38 percent), data lakes (33 percent), and wearables (33 percent) are gaining traction in day-to-day operations.

The draft guidance outlines that RWD needs to be of sufficient quality and integrity to support regulatory decision-making and align with agency expectations for sponsor and investigator conduct. Regulators consider RWD to have integrity when it is both "relevant and reliable." To be relevant, the data should have sufficient detail

to be analyzed using sound statistical techniques and interpreted using solid scientific judgment and methodologies. Relevant data in clinical trials can include patient characters, exposures, and outcomes.

In terms of reliability, data collection practices are crucial. We need to look at whether the processes in place during data collection and analysis are robust so that errors are minimized and data quality and integrity are sufficient. Requirements for source data verification need to be clearly specified. Depending on device/data sources, verification methods will vary. Some RWD sources (such as wearable devices and electronic clinical outcome assessment patient diaries) require careful consideration, whereas others (such as electronic health records and disease registries) are a little more straightforward and capable of capturing evidence directly from source documents.

Not all RWD is created equal and, by itself, the information is not sufficient to generate the evidence the FDA needs to support a given regulatory decision. RWD's relevance to quality and patient safety depends on where it comes from, how it is managed, and how well it is analyzed. As this guidance becomes institutionalized into pharma's compliance and risk strategies, it is becoming clear that regulators' ultimate goal is to effectively reduce the risk of bias in data source collection and analysis – and that should be the industry's goal as well.

Patient-Centric Perspectives

Patient-centricity needs to be more than a buzzword. Here's how Boehringer Ingelheim is creating a culture of patient-centricity throughout the entire organization.

By Keri Yale, Head of Patient Centricity & Engagement, Boehringer Ingelheim

Early in my career, I worked with the HIV/AIDS community as an advocate liaison. Their constant refrain was "information is power," and it's something that remains true to this day. If patients are active in their healthcare journeys, they are able to make the right decisions and more likely to proactively seek out better options. And for those of us in the pharma industry, information shared in the conversations with patients and caregivers empowers us to develop products, services, and solutions that provide even greater value to the patient community. Patients are uniquely placed to share what it is like to live with the condition, and we should be prepared to listen and take action.

Not only is robust and early patient engagement considered best practice, it is now incumbent on pharmaceutical companies to help discover what matters most to patients and to communicate insights gained from patients to regulatory authorities. In the US, the 21st Century Cures Act helped propel the field of patient experience data in regulatory decision making, and both companies and agencies need to continue to collaborate in this culture change to align with what could be described as the "new bedside manners for the 21st century."

As patient-centricity has gained traction across the health ecosystem, it has at times been referred to as a buzzword. To ensure it doesn't remain just a buzzword, patient-centricity should be a way of thinking and acting with authenticity. It should sit at the core of everything we do. At Boehringer Ingelheim, patient-centricity is a mindset and culture that puts patients first. Growing beyond buzzwords and making its value known gives it a real meaning within our organization, and it has been paramount to our success.

However, there have been challenges in embedding patient-centricity throughout the company. We knew there were pockets of excellence and several people working in this way, but we wanted to make it part of the very fabric of our company. How? We made each senior leader responsible for having a patient-centricity plan to identify the objectives and activities for the department they lead. These plans looked very different from one team to the next; we want them to be authentic to the nature of the work in each department. I believe it's critical in any company to have "buy-in" from its leaders when it comes to patient-centricity. If leaders are talking about patient-centricity, it emphasizes the importance of the topic and employees are more likely to get on board.

We have also created a patient ambassador program. Patient ambassadors are champions of patient-centricity (I refer to them as our "boots on the ground"). These individuals are either nominated or self-identify, and they play an active part in advancing our patient-centric culture. They participate in monthly meetings, which include knowledge exchange opportunities and help identify new ways to address patient-centricity within their own teams. After onboarding, the monthly meetings offer insights into best practices and guidance on how to incorporate patient-centricity into their

roles – and their priority is to champion a patient-centric mindset in themselves and their teams.

I'd also like to mention our "Promise Tree." It starts with a bare tree trunk. Employees are asked to take a leaf and write the first name of someone they know living with a disease and their relationship to that person. They place the leaf on the tree and make a promise to that person that they will continue to contribute to the health of patients. This exercise reminds us that when we say patients, we mean people. Friends, parents, siblings, neighbors. The Promise Tree connects everyone to that individual experience of health and helps build an understanding of what it is like to live with a condition or illness.

Another tool we use is Patient Minutes – short presentations that highlight the story of somebody living with a disease or a patient engagement activity that helps us better understand patients' needs. It could be patient stories, patient insights, or information we want to share broadly so everyone can benefit from that knowledge. Hearing directly from patients is our most powerful tool. It helps to better understand their experiences and reminds us of the urgency and the importance of our jobs. With this tool (and others), patient ambassadors and other employees are able to amplify patient-centricity.

We've been engaging with patients for decades – beginning with our work in HIV/AIDS, but, over time, we've needed to shift the mindset from not just appreciating that we can learn from patients, but being strategic about it, giving it a budget, and making it a genuine priority. We've had to think about what information we capture – and how we capture it. We've also had to ask, "What are we learning?" What is the value that is being delivered to the business and, more importantly, to patients? Could it be shown in trial design, materials, education, or how we create awareness?

Ultimately, I'd like to see patients getting more involved in their own healthcare – and in the development of new medicines and therapies. As an industry, we've expanded the ways through which we gain insights and have learned much about how to involve patients from the very beginning. Understanding patient experience and preference, as well as addressing unmet needs, should shape the development of products and services – not the other way around. By developing our products and services in collaboration with patients, both the patient community and the industry stand to succeed.

A more patient-centric mindset in an organization doesn't happen overnight. It takes activities. It takes people. It takes a change in thinking. It's not just something you talk about or something you're doing, but a way of being that flows from the leadership down and right back up through an organization that has become newly empowered and energized because its employees know that they are partners in this change. The patients we serve are why we get up in the morning and why we maintain a strong sense of purpose and urgency.

The Dangers of Dengue

Dengue is a deadly, climate-sensitive disease that is spreading rapidly in tropical and subtropical climates worldwide. And because there is no specific cure, we need more investment in new treatments

By Neelika Malavige, Head of Dengue Global Programme at the Drugs for Neglected Diseases initiative

Did you know that dengue is the most rapidly increasing mosquito borne infection worldwide? In the last few months, countries on almost every continent have faced large outbreaks. Peru is now experiencing its worst dengue crisis on record, while Bolivia, Bangladesh, Nepal and Pakistan went through devastating outbreaks in recent months. In 2022, hospitalizations were several times higher than hospitalizations for COVID-19 in many endemic countries. Worse still, the incidence of dengue further increased in early 2023.

When I started my career as a medical doctor, I witnessed the devastating impact dengue had on patients firsthand, and for the last 20 years I've dedicated myself to finding a treatment for the disease. Although the majority of dengue infections cause asymptomatic or mild illness, a proportion of infected individuals develop serious complications, including plasma leakage, which can lead to shock, bleeding manifestations, organ dysfunction, and, in some cases, death.

The lack of treatment options for dengue is a major cause for concern because climate change and rapid urbanization are causing the disease to spread further and faster. In 2019, dengue was named one of the top ten

threats to global health by the World Health Organization. In fact, dengue incidence has increased eightfold over the past three decades, while the age standardized disability adjusted life years (DALYs) increased by 107.6 percent. Unfortunately, dengue is mostly endemic in low- and middle-income countries and there is little financial incentive for commercially driven pharmaceutical research. Moreover, four serotypes of dengue exist, which makes the development of treatments and vaccines even more difficult.

Despite these challenges, progress has been made; thanks to a better understanding of disease pathogenesis and clinical progression, doctors have managed to decrease casualties through strict monitoring and careful fluid management. Currently, all patients with suspected dengue are monitored at least once a day in outpatient facilities or primary healthcare settings; daily blood samples and blood counts allow us to detect lowering platelets and rising haematocrits, which is suggestive of plasma leakage.

Patients suspected of having fluid leakage or who develop any of the dengue warning signs – mucosal bleeding, persistent vomiting, and abdominal pain – are admitted to hospital. And those at risk of developing severe disease, such as pregnant women, those with comorbidities (for example, diabetes, obesity, and hypertension), and the elderly, need to be monitored several times a day. This comprehensive approach has reduced the case fatality rates of hospitalized patients from around 5–10 percent in the 1980s and 1990, to below 0.1 percent in many countries today, such as Thailand and Indonesia. But these monitoring measures are only feasible when patient numbers are low; in large outbreaks, such efforts are unsustainable.

Vector control efforts have been made in numerous countries; for example, using fogging to reduce the number of Aedes mosquitoes that carry the disease. Though novel techniques, such as the World Mosquito Program's Wolbachia method, are likely to improve results, this approach alone is not enough. Several dengue vaccines have also been developed, but they are not equally effective against all serotypes of the disease. For example, the TAK-003 vaccine looks promising and is currently being registered in multiple territories; however, it shows less efficacy against dengue serotype 3 in "dengue naïve" individuals.

According to some predictions, the number of people at risk of dengue will reach 60 percent of the world's population by 2080, partially linked to rising temperatures instigated by climate change that allow Aedes mosquito larvae to mature earlier, increasing biting frequency and transmission correspondingly. I have seen many experts disregard the impact of mild dengue. But dengue, even in its so-called "mild" form, presents huge economic repercussions. The estimated cost in India alone in 2016 was the equivalent of around $5.71 billion,making a disease with one of the largest enormous economic tolls. Dengue is no longer affecting only historically endemic countries, but also new geographical locations including high-income countries previously unaffected by the disease. Notably, both France and Spain experienced unprecedented locally transmitted dengue outbreaks in 2022.

Similar to the global effort against COVID-19, we must use multiple strategies, join hands, and invest in research to develop therapeutics for dengue – the disease the world can no longer afford to neglect.

References are available in the online version of this article

Creating for Tomorrow

Asahi Kasei Bioprocess – based in Chicago and owned by a Japanese company – supplies bioprocess equipment, consumables, and scientific support, and has always considered itself to be a future-facing company. With the biopharma industry increasingly realizing the importance of sustainability, Asahi Kasei Bioprocess is putting its best foot forward. We interview Clayton Weber, Systems Engineering Lead/ Sustainability Lead at Asahi Kasei Bioprocess, to learn more.

What is your role at the company?

I started my career with Asahi Kasei Bioprocess as a project engineer, but today I am the systems engineering lead, which means I get involved with almost every piece of equipment our company makes. I assist with kicking off discussions with the customer to understand their needs and timelines; we'll then go through full spec, procurement, assembly, and testing of the equipment until it is ready to be delivered to the customer. I'll then be involved onsite with training and set up.

I've built chromatography columns, chromatography systems, inline buffer formulation skids, virus filtration systems… a wide range of equipment. Right now, there is a strategic focus on oligo synthesis, and customers are also looking to update bioprocesses to be more efficient and cost-effective; for example, by moving to automated inline buffer formulation systems, which reduce cleaning and the amount of chemicals used – and get companies away from using "tank farms." Ultimately, adopting these kinds of processes can help companies reduce their environmental footprints.

What does sustainability mean to Asahi Kasei Bioprocess?

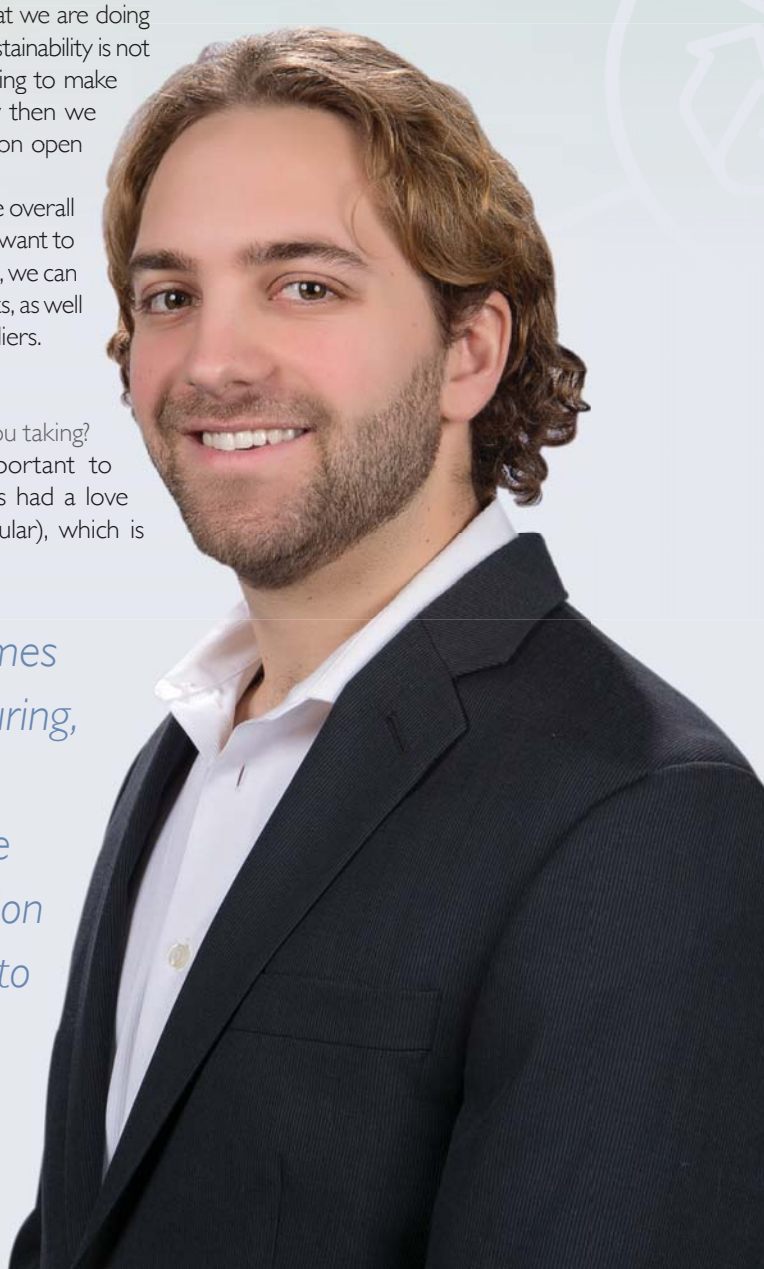
Sustainability is a growing trend in the pharmaceutical industry. We've always been focused on pioneering the biologics of the future by developing the equipment needed to manufacture them. Personally, I think the industry's future is brighter thanks to increased focus on efficiency and sustainability, but there is still much to do. Most biopharma companies are now establishing sustainability programs and targets – and asking their vendors to do the same. At Asahi Kasei Bioprocess, we regularly receive enquiries about what we are doing on the sustainability side. Sustainability is not proprietary and if we're going to make a difference as an industry then we need to keep communication open and share success stories.

We are a small part of the overall Asahi Kasei Group, but we want to lead by example. In doing so, we can influence other business units, as well as our customers and suppliers.

As part of your role, what sustainability actions are you taking?

Sustainability is very important to me personally (I've always had a love of the outdoors in particular), which is why I am delighted to have stepped up as "sustainability lead" within the company. Sustainability can be broken down into different areas including environmental, social, and economic. However, my role focuses primarily on the environmental side. For now, we are focusing on three core areas: the emissions and waste that we produce as a company and that is directly under our control; emissions and waste caused by our products; and external emissions, such as those produced by vendors (remember – if your vendors are not sustainable then it can affect your own

"When it comes to manufacturing, we are also increasing the implementation of single use to be more sustainable."



AsahiKASEI BIOPROCESS

company and the carbon footprint of your products and services).

The first two areas are easier to address because it is about looking at your own company and what you can do – and they are within your control. To help reduce our emissions, we have signed a contract for a solar initiative. We have a lot of roof space and we have calculated that we will be able to offset 100 percent of our energy consumption – a phenomenal first step, which will also save substantial energy costs.

We have also looked at our waste management. We receive a lot of boxes and packaging materials, which can be recycled. Recycling is commonplace, but do not assume that this is already happening at your company, even if you already have recycling bins around your offices. It is important to find out what your waste team is actually doing. In fact, I recommend this as a good starting point for any sustainability strategy. At our company, I discovered that our waste team was disposing of everything as general waste. We took swift action to resolve this, and we now have a specific company that manages the waste and recycling. Due diligence and conversations can make a big difference!

When it comes to manufacturing, we are also increasing the implementation of single use to be more sustainable. There is a perception that single use components have a negative impact on sustainability, due to the disposal of consumables; but they can actually help reduce cleaning needs (less water, less energy, and less chemicals). We are now looking into how single use can be recycled and disposed of appropriately – and some single use tubing manufacturers are also taking a stand to discuss solutions.

What are your top tips for other companies looking to implement sustainability initiatives?

You can choose how far to take your recycling scheme, but it should be tailored to the type of waste that your company produces. Recycling cardboard, plastic, and bottles are easy wins; but there are also companies that handle other materials, such as polystyrene and foam. Just keep in mind, you may not be able to identify a single stream for your recycling, which can complicate matters. I advise seeking recommendations from companies that have similar waste outputs, or even your current waste management company. In our case, we looked online, through sustainability forums and articles for perspectives, and we also talked to our sister companies, which ultimately secured us a good deal with a company that could handle six different categories of waste. The important thing is to ensure proper disposal.

How can companies support their vendors?

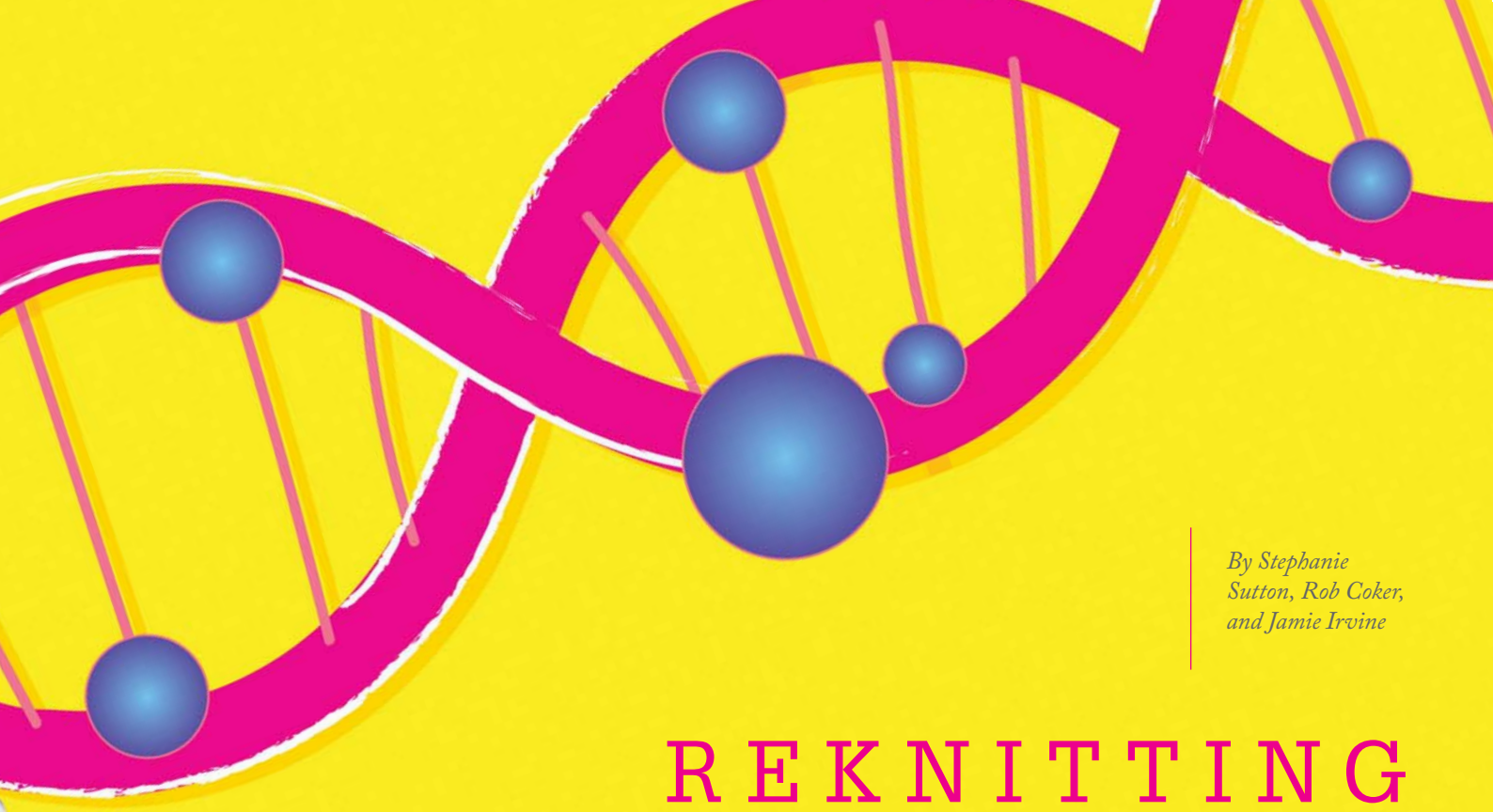
As noted previously, some pharmaceutical companies are now requiring their vendors to have sustainable programs in place. This trend will likely continue, pushing more companies to introduce sustainability strategies with more concrete measures and to think bigger about their efforts.

We believe we have a responsibility to help our vendors with their sustainability goals – particularly the smaller companies that do not have the same resources as larger businesses. We've sent surveys to our vendors to understand who is ready to ally with us on sustainability. Not everyone is going to get on board straight away – and right now we certainly don't have all the solutions – but by starting conversations with vendors we can push the issue to the forefront to find a path forward. For example, tube set manufacturers may not yet be looking at sustainable sources for their raw materials, but if they are willing to explore this option, it is a good first step. Any partnerships in this area may also inspire additional partnerships further down the line.

What are the challenges that the industry faces in being more sustainable?

One of the biggest challenges in sustainability for the biopharma industry will be embedding it throughout whole supply chain – from procurement of raw materials all the way to recycling. We don't yet have all the answers, but people are trying different approaches. Collaboration will be required to get initiatives across the board. There are a lot of vendors out there, and if a vendor refuses to engage in finding sustainable solutions, then ultimately, they run the risk of being replaced.

At Asahi Kasei, we like to say, "As the world constantly changes, we will continue to contribute to life and living for people around the world by Creating for Tomorrow." The pharma, bioprocess, and biotech industries all contribute to life and living, but we must go beyond therapeutics. You can't say that you are contributing to life and living in one aspect, but then refuse to contribute to sustainability efforts and reducing the waste and harm produced on an industrial scale. We've already made big progress at Asahi Kasei Bioprocess – with our work on solar panels and the partnerships with vendors being some of our biggest sustainability success stories to date. But there is more to come. Sustainability is not going to stop; in fact, the emphasis on sustainability will only increase – and, in time, it will move beyond emissions and the environment to other aspects, such as community. Whenever we finish one goal at the company, we'll have another one right behind it. I have a great team to support me and what we can achieve is an open book. We will continue to support the biopharma industry by leading the way in both technology and sustainability.



By Stephanie Sutton, Rob Coker, and Jamie Irvine

REKNITTING *the Fabric of Life*

Someone's famous uncle once said,
"With great power comes great responsibility."
Genome editing is an incredibly powerful technique with huge promise and potential for medicine, as well as many other fields including agriculture and the environment. But those wielding the power must cut through the hype, evaluate the potential, and use it wisely. Here, we talk to a selection of experts using genome editing and CRISPR/Cas9 for drug development purposes to get their views on the field.
"A new genetic revolution."
"The ultimate therapy."
These are just two of the tantalizing phrases used by our panel when describing the technology and what it could accomplish…

WHY ARE YOU SO EXCITED ABOUT THE POTENTIAL OF GENE EDITING?

LDJ: CRISPR/cas9 is a captivating technology. In 2019, the first American patient treated with CRISPR technology for sickle cell disease achieved disease-free status. This technology's potential also extends beyond medicine; it has the power to address food crises, improve agriculture, aid drug development and pathogen detection, and offer solutions to climate change. CRISPR technology has made re-writing the code of life easy, accurate, and accessible, fueling a new genetic revolution.

TC: It is such an exciting area. Changing a fundamental aspect of biology and permanently correcting genetic messages with a level of elegance that was previously unthinkable. If diseases were created by nature, then now we have the ability to challenge them using tools presented to us by Mother Nature herself. Gene editing could be the ultimate therapy for targets that have previously been undruggable.

RH: For me, it's about opening the door to what I think of as the third leg of the stool in the world of drug development. Today, that stool is a bit rickety with just two legs: small molecules and antibody/protein therapies. I believe the third leg is genetic medicine. Genome editing is important because if we can manipulate the genome, either ex vivo or in vivo in a variety of contexts, then we will be able to help so many different kinds of patients with different diseases.

ER: Ever since the completion of the human genome project, and in the years following, the scientific community has accumulated a massive amount of sequence data. What was initially lacking was the ability to actually manipulate that information in cells. Gene editing is the tool that allows us to utilize that information in the context of a living system to better understand pathways and how those sequences interact and are controlled. An analogy I like to make is to consider the genome a database of information; the cellular machinery is the software that runs the programs; and gene editing is a programming language we can use to manipulate the data and run programs. The introduction of CRISPR has also been a revolutionary step in making genome editing applications available to everyone, no matter what organism they may be working with.

HOW DO WE SEPARATE THE HYPE FROM THE REALITY?

LDJ: It is essential to approach CRISPR technology with critical thinking and a balanced perspective. While acknowledging its incredible potential as a powerful gene editing tool, it is equally important to recognize that the field is still in the early stages of development and faces significant challenges. Researchers worldwide are working to overcome these obstacles, and though wide-scale implementation of CRISPR applications, particularly in clinical settings, may take time, the technology continues to demonstrate hope.


Tirtha Chakraborty

TC: The hype and excitement will help to fuel interest and further research in the field. However, we are not yet at the epicentre of precision genome engineering for most cells in the body, so while there is hype, we need to be careful about what is truly attainable with today's technology and what is not. We still cannot get to every part of the body, even with existing delivery technologies for in vivo genome engineering. Most of the focus to date has been on the liver. How do we target the lung? Or neurons? Or even the skin? We can't do this regularly just yet. If a delivery technology emerges that is capable of reaching every part of the body, without tissue off-target effects, then we will have a real victory. Additionally, most cells in our body do not divide. Today's standard CRISPR/Cas platform is not very good in the precision engineering of those cells. That needs to be resolved as well, if the potential of this technology is to be fully harnessed.

The negative side of the hype is that there are some people out there who believe we're going to make CRISPR babies every day. If you want to change the world, you need to be cautious. We need to work very closely with regulatory authorities about what is possible and what is not, as well as to understand what the implications are when we're working with humans rather than mice.

RH: We can all be guilty sometimes of creating hype. Scientists have pointed out that there are around 7,000 monogenic genetic diseases. Wouldn't it be great if you could use CRISPR to address each of them? While certainly true at the 50,000 foot view level, I think the practical reality of how you do that becomes complex quickly. Sickle cell disease is a classic example where one mutation is shared across all patients, which opens the door to one genome editing strategy that could potentially serve the entire patient population.

In diseases like cystic fibrosis or muscular dystrophy, patients have different mutations that may require a multiplicity of different kinds of genetic medicines. And that's just the tip of the iceberg. There is definitely a lot of potential, but we have a lot of work to do.

However, I always get worried when I talk to friends who are not in the biotech world and who base their reading on mainstream news; some of them have come to the conclusion that you can CRISPR any gene in any cell at any time. That is not reality – nor will it be anytime soon. We have to be very cautious about the kinds of promises we make to patients and to our communities about what is actually possible today, what we hope will be possible tomorrow, and what some futuristic landscape might look like.

The reality today is that there is a fairly short list of cell types, either outside or inside of the body, that we can edit with high fidelity in a way that could lead to near-term clinical translation. However, there's a lot of work happening that will open the door to additional tissues and cell types in the not too distant future.

ER: The hope has always been that once a genetic mutation leading to disease was known, genome editing might be applied to repair the mutation and lead to a cure. But from a therapeutic reality perspective, this is not as easy as it sounds. The main challenge for gene editing remains the matter of delivery. If the mutation requires only a small portion of cells to be targeted for delivery and editing, there is a good chance that gene editing can play a role, but with most diseases this is often not the case. I frequently receive letters from desperate parents whose child has been diagnosed with a disease associated with a genetic mutation asking if CRISPR can be deployed to help their child, but often the situation would call for editing of virtually all the cells in the body, and this just isn't possible at this time.

WHAT HAVE BEEN THE BIGGEST MILESTONES TO DATE?

LDJ: Since the 2012 discovery of the CRISPR-Cas9 system by Nobel laureates Jennifer Doudna and Emmanuelle Charpentier, followed by Feng Zhang's demonstration of its

The Experts

Tirtha Chakraborty, Chief Scientific Officer, Vor Biopharma

"Vor Bio is the first ever company in the world doing allogeneic genome engineering for metabolic transplants. We are focused on the treatment of hematopoietic diseases and making the hematopoietic transplant the best type of transplant on the planet."

Rachel Haurwitz, CEO at Caribou Biosciences

"Caribou Biosciences is a genome editing company that was spun out of Jennifer Doudna's lab at the University of California, Berkeley. Caribou has invented its own next-generation CRISPR technology, called chRDNA (CRISPR hybrid RNA-DNA), which we believe is more specific than first generation CRISPR/Cas9. The chRDNA genome-editing technology is being used to develop allogeneic cell therapies – mainly for oncology applications. Our pipeline includes CAR-T cell therapies for hematologic diseases and iPSC-derived CAR-NK (natural killer) cell therapies for solid tumors."

Linda De Jesus, Vice President and General Manager, Global Head of Commercial at Integrated DNA Technologies (IDT)

"IDT is a biotechnology company that specializes in the manufacture and development of custom nucleic acid products. We also consider ourselves to be on the cutting edge of gene editing technologies. We've helped enable significant contributions in the gene editing field by providing complete CRISPR genome editing workflow solutions – from design to analysis – through our CRISPR systems."

Eric Rhodes, CEO at ERS Genomics

"Our founder, Emmanuelle Charpentier, won the Nobel Prize in 2020 for the discovery of CRISPR/Cas9. At ERS Genomics, we are in the business of licensing her CRISPR intellectual property for commercial use. We manage the rights outside of use in cell and gene therapy. We therefore deal with a broad cross-section of companies and industries, from basic life science research to animal health, to industrial applications."

In the Pipeline

Vertex and CRISPR Therapeutics

These two companies have been collaborating on CRISPR/Cas9 since 2015. Their treatment for sickle cell disease (exagamglogene autotemcel; exa-cel) is being watched closely. Exa-cel is under priority review by the EMA and FDA. This is the closest gene-edited therapy to a potential approval.

Exa-cel is an autologous, ex vivo CRISPR/Cas9 gene edited therapy which involves editing a patient's hematopoietic stem cells to produce high levels of fetal hemoglobin in red blood cells.

Beam Therapeutics

Beam Therapeutics is also working on treatments for sickle cell disease. The company uses base editing technologies developed at Harvard University and the Broad Institute of MIT and Harvard, and is working on a range of therapeutic candidates, including three undisclosed targets with Pfizer. Its lead programs are Beam-101 and Beam-102 – both are ex vivo gene editing therapies for sickle cell disease.

Its Beam-101 candidate produces base edits to activate fetal hemoglobin; Beam-102 edits the causative hemoglobin S point mutation to recreate a naturally occurring normal human hemoglobin variant, HbG-Makassar.

Editas Medicine

Another company with its eyes on sickle cell disease, Editas Medicine's lead clinical program is EDIT-301, an ex vivo gene editing therapy for sickle cell disease and transfusion-dependent beta thalassemia where patient-derived CD34+ hematopoietic stem and progenitor cells are edited at the gamma globin gene promoters.

The company also has a partnership with Bristol Myers Squibb to develop ex vivo gene edited cell medicines for cancer.

successful use in mammalian cells in 2013, several significant milestones have been achieved. Noteworthy achievements include (but are not limited to) the first ex vivo CRISPR therapy for sickle cell disease, the first clinical data supporting safety and efficacy in in vivo CRISPR genome editing for transthyretin amyloidosis, and the development of prime and base editing technologies for precise genome modifications.

TC: We have seen some promising genomic engineering using lentiviral and AAV vectors, but they have their limitations regarding both safety and efficacy. Using CRISPR, a much more sophisticated form of genome engineering has reached the clinic and demonstrated real benefits in patients already, such as CRISPR Therapeutics' and Vertex's programs for beta thalassemia and sickle cell disease. The in vivo editing success by Intellia Therapeutics is also very impressive.

Vor Bio has been working to perform allogeneic genome engineering for hematopoietic transplants. So far, the engineered hematopoietic transplants are all autologous, and while they are wonderful, nobody has done this with healthy donor derived allogeneic cells and for malignant diseases. In the future, I really hope that engineered allo-transplants for cancer will become the standard of care.

RH: There have been exciting early clinical datasets from a few companies – and I'm proud to say that Caribou has contributed to that! It is remarkable to think about how quickly this field has moved. It was the summer of 2012 when some of my co-founders published what has turned into a seminal manuscript in Science, demonstrating that you could reprogram the genome using Cas9 and CRISPR all-RNA guides. And here we are, not quite 11 years later, with a number of different organizations who are developing initial clinical data. We could even see the first approved CRISPR medicines sometime later this year.

ER: Several promising CAR-T approaches for treating cancer utilize genome editing. CRISPR Therapeutics' sickle cell and beta-thalassemia trial results indicate both safety and efficacy. Intellia's ATTR program using a systemic delivery approach is also highly encouraging. One of our licensees recently presented data on engineering of mushrooms intended for clinical studies, opening up another important avenue of new drugs.

WHAT ARE THE ETHICAL QUESTIONS AROUND GENE EDITING?

LDJ: Germline editing, exemplified by He Jiankui's controversial work in 2018, raises concerns about the long-term implications

Rachel Haurwitz

and unforeseen consequences of modifying the human genome. Since the twins' genomes were modified while they were still embryos, the created genetic change can be transmitted to their children. Other ethical debates center around issues such as creating "designer babies" or non-medical genetic enhancement that could exacerbate existing social inequalities. Cultural and religious differences may also influence attitudes and ethical considerations surrounding CRISPR genome editing.

Thoughtful evaluation of ethical considerations, facilitated by collaboration among scientists, regulatory bodies, and policymakers, will be necessary to ensure responsible and informed decision-making for all, including those in underdeveloped and developing nations.

RH: Absolutely there are ethical implications – and Caribou has been involved in some of those discussions both in the US and internationally. I was one of the first industry speakers to participate in the latest iteration of the International Summit on Human Genome Editing, where academic, government, and industry leaders gather to discuss advances in the technology and the responsibility the scientific and biotechnology community has in ethically implementing this technology.

This is a tremendous technology, and it comes with a tremendous responsibility to be ethical stewards for its appropriate use. For us, there is a very firm line when it comes to embryo editing. We have a company policy that we do not edit human embryos. Period. The end. We bake this into license agreements with other companies too. If you happen to buy an RNA reagent from a company like IDT, for example, you will find a document in the box that is a limited use label license that says you cannot use this reagent for human embryo editing.

TC: I agree; we are not ready for CRISPR babies. For pretty much everything else, I think there is some concern but we should not worry too much. The first people who need to understand CRISPR are the doctors who are going to allow these trials – because all of this good work will hit a brick wall if clinical investigators are not informed enough. There are still some questions about CRISPR, mostly due to lack of familiarity with a new field that has the

power of making permanent genetic changes, but we must be brave. We don't want to see patients die without therapies because we weren't brave enough to try something new.

ER: As with any emerging technology, there are clearly many ethical issues that need to be addressed. The CRISPR babies triggered many discussions surrounding genome editing in embryos. Here, the questions are about the long-term consequences of making permanent changes to the human gene pool. But so far, there hasn't really been a thorough global discussion on the topic or formally adopted guidelines. We also need to consider the topic of enhancement versus therapy; CRISPR/Cas9 has the potential to go beyond treating genetic diseases and enable genetic enhancements or modifications for non-medical purposes. There are many questions about the ethics of using gene editing to enhance traits such as intelligence, athleticism, or appearance. Should CRISPR/Cas9 be used for these purposes? What are the limits and consequences?

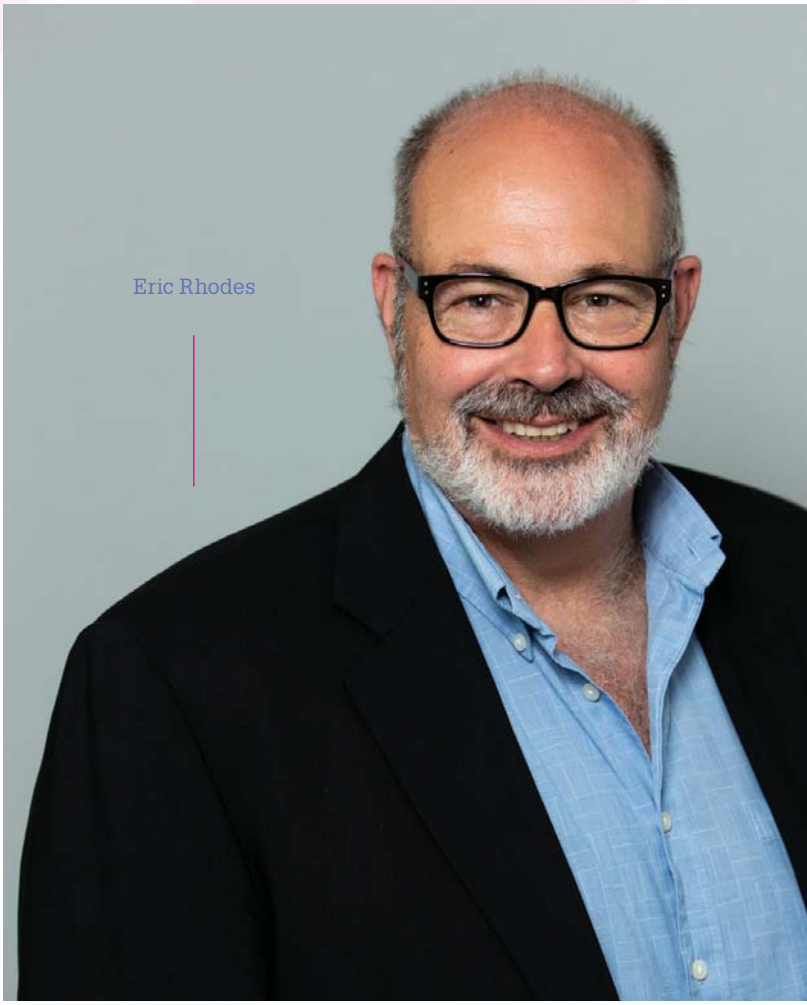
And then what about access and equity? Could CRISPR/Cas9 exacerbate existing inequalities if it's only available to those who can afford it?

A final area of concern I will mention is the use of gene drives. Used safely and ethically, these can be a tremendous tool, but they also represent a very clear danger if misused. Public debate, interdisciplinary discussions, and the involvement of stakeholders are essential in navigating these complex issues as we move forward with this genuinely revolutionary technology.

WHAT ARE THE BIGGEST CHALLENGES FACING THIS AREA OF THE INDUSTRY?

LDJ: There are several crucial questions in the field that scientists are actively addressing. These include concerns about off-target effects, where unintended editing occurs in regions of the genome similar to the target region. Additionally, efficiently delivering CRISPR reagents in a cell type- and tissue-specific manner remains challenging. Evaluating the long-term effects and ensuring the safety of CRISPR-based therapies are also essential. Comprehensive long-term studies, both preclinical and clinical, are necessary to assess gene editing stability, potential immune responses, and any unintended consequences resulting from genome alterations.

RH: Not every underlying technology is going to be the best fit for every disease. For any given disease, we have the responsibility to figure out what is the best collection of technologies needed that could develop the right therapy.



Eric Rhodes

At Caribou, we have been focused on off-the-shelf cell therapies for oncology, and use our genome editing capabilities to do what we call "armoring" to enhance the cells and make sure they have sufficient antitumor activity, which is needed to rival that of today's approved autologous CAR T therapies.

We believe that off-the-shelf has to be the answer if we want to deliver these kinds of therapies to increasingly broad patient populations. But it's not as easy as taking a healthy T cell from a healthy donor and adding a CAR, which would be foreign to the patient's immune system and thus rejected. We have to enhance, or armor, the cells to bridge the gap.

ER: I think concerns remain around safety and which version of genome editing might be the safest to use in each clinical situation. Base editing and prime editing are both seen as potentially safer versions of CRISPR, but both have limitations that don't make them as broadly applicable as the

A Brief History of CRISPR

Few breakthroughs have captured the imagination like CRISPR in the field of genetic research. Short for "Clustered Regularly Interspaced Short Palindromic Repeats," CRISPR DNA sequences were first located in Escherichia coli bacteria in 1987. Yet, unbeknownst to its early investigators, the true origin and significance of this discovery would remain a phenomenon for some time. Fast forward to 1995, Francisco Mojica from the University of Alicante found similar structures in the archaeal genome of Haloferax mediterranei. Upon noticing the similarity of the elements he described in archaea with previously known DNA repeats in bacterial genomes, Mojica hypothesized that CRISPR loci include fragments of foreign DNA, and were related to the immune system of bacteria and archaea.

Building upon Mojica's seminal findings, subsequent research revealed that bacteria possess the ability to transcribe specific DNA elements into RNA as a responsive measure to viral infections. These RNA molecules act as guiding beacons, leading a specialized nuclease named "Cas" (short for "CRISPR-associated") in a sophisticated defense mechanism against invading viruses. More specifically, Cas proteins precisely cleave foreign DNA, incorporating the resulting fragments into CRISPR arrays – continuous DNA stretches. Separate Cas proteins then facilitate the expression and processing of CRISPR loci, which generate CRISPR RNAs (crRNAs). crRNAs serve as guides for Cas nucleases, directing them to exogenous genetic material containing a species-specific protospacer adjacent motif (PAM). The CRISPR complex finally binds to the foreign DNA and cleaves it to destroy the invader. To date, CRISPR repeats have been identified in the majority of archaeal genomes and nearly half of the bacterial ones examined thus far.

Of all known Cas proteins, the most studied are those belonging to the system of directional cutting of foreign DNA, which includes the nuclease Cas9. CRISPR-Cas9 offers the advantage of simultaneously targeting multiple genes, eliminating the need for separate cleavage enzymes. Moreover, the system can be easily combined with customized "guide" RNA (gRNA) sequences, which are readily accessible to researchers. This understanding paved the way towards a major advancement in CRISPR genome editing technology: homology-directed repair. Enabling precise integration of donor DNA where the cut site occurred, this technique allows for activation of gene expression. Researchers have since permanently modified genes in living cells and organisms and, in the future, may be able correct mutations at precise locations in the human genome to treat genetic causes of disease.

> "THE POWER TO ENGINEER BIOLOGICAL SYSTEMS AND ORGANISMS COMES WITH INHERENT ETHICAL CONCERNS"

However, the power to engineer biological systems and organisms comes with inherent ethical concerns. Editing the genomes of gametes and early embryos raises profound implications, not only for the individuals but also for future generations, since there is a potential for not just curing diseases, but also for enhancing desirable traits. As a result, the scientific community has encouraged a moratorium on human germline editing until a comprehensive understanding of the ethical implications and societal consequences is achieved. In many countries, it is illegal to genetically modify human embryos for purposes other than reproduction.

From its humble origins, to its elucidation as a remarkable immune defense system, CRISPR has opened doors to a realm of possibilities for manipulating the fundamental aspects of life. Its applications extend across medicine and biotechnology, offering the potential to revolutionize therapeutic interventions beyond our previous expectations. As scientists continue to explore and harness the power of CRISPR, it will be crucial to strike a balance between scientific progress and the responsible consideration of ethical implications.

Enhancing CRISPR Cas9

Is safeguard sgRNA the key to reducing the off-target effects of gene editing, while increasing its potential applications?

> "ADOPTING SGRNA AND EXPANDING THE FUNCTIONALITY OF CRISPR TOOLS WILL INCREASE SAFETY AND ACCESSIBILITY FOR EACH DISEASE"

In Japan, researchers at Kyushu University and Nagoya University have been working on reducing issues with mutations (and hopefully toxicity and side effects) when using CRISPR Cas9.

Masaki Kawamata, assistant professor at Kyushu University and one of the authors of the study, says, "Even before the discovery of the CRISPR editing system, I was interested in genome editing. I have actually produced genetically modified mice and rats using knockout and knock-in strategies. When I joined Fernando Camargo's lab at Harvard University in 2013, he asked me to start working on gene therapy by establishing the CRISPR Cas9 system in the lab."

Kawamata and colleagues were interested in the current limitations of CRISPR Cas9. If CRISPR Cas9 activity is too strong, off-target effects and cytotoxicity can be introduced. Even on-target, excessive DNA breaks lead to large deletions as well as chromosome loss and translocations. "In addition, the strong Cas9 activity prevents precise genome editing, such as mono-allelic single nucleotide replacement, which is important for generating and correcting disease models," says Kawamata. "We have overcome these problems with a new technology that allows us to fine-tune the activity of Cas9. However, CRISPR Cas9 itself still has its limitations for precision gene therapy in that it preferentially introduces indels that may cause unknown side effects."

Publishing in Nature Biomedical Engineering (DOI: 10.1038/s41551-023-01011-7), the authors reported on a "renovation strategy" to directly limit Cas9 activity through simple and tunable single-guide RNA (sgRNA) redesign – what they refer to as safeguard sgRNA in the paper. Kawamata explains how the approach works. "Addition of cytosine stretches to the 5'-end of conventional sgRNAs enables length-dependent and large-dynamic-range inhibition by constraining the formation of functional Cas9 complexes. Overall, short cytosine extension reduced cytotoxicity and enhanced homology-directed repair, while maintaining bi-allelic editing capacity. Long extension further decreased on-target activity but improved specificity, thereby facilitating mono-allelic and precise editing."

The safeguard sgRNA is also compatible with CRISPR activation/interference and Cas12a systems. The integration of a fluorescence-based allele-specific indel monitor system (AIMS), computational simulation, and systematic validation established optimal windows of Cas9 activity for diverse applications, including precise one-step generation and correction of disease-associated single-nucleotide substitutions. "Importantly, this method dramatically alleviated p53 activation and cytotoxicity in highly sensitive human pluripotent stem cells, enabling precise gene correction. Together, the safeguard sgRNA represents a promising strategy to prune excessive activity, improve safety, and maximize applicability for CRISPR-Cas9-related biomedical technologies," says Kawamata. "Safeguard sgRNA is expected to be safer and more efficient than the conventional gRNAs that have been used in clinical trials. Since the modification is simple, requiring only the addition of cytosine to the 5' end of the gRNA, safeguard sgRNA can be applied to clinical trials relatively easily without major changes to the protocol. Adopting sgRNA and expanding the functionality of CRISPR tools will increase safety and accessibility for each disease."

The research team initially applied the safeguard sgRNA in fibrodysplasia ossificans progressiva (FOP) disease model as a proof of technology because they believe that gene-correction therapy has strong potential in the area. Based on the technology, a start-up company called One Genomics was founded in the US. The focus is developing safe and high precision novel gene therapies using safeguard sgRNA technology.

Linda De Jesus

more traditional CRISPR/Cas9.

I also believe that further discussion on the ethical concerns of genome editing must be clearly a priority. Making gene editing therapies affordable and broadly available will also be a challenge for the industry in the coming years. For my company, our goal over the next decade is to expand the use of CRISPR/Cas9. We want more companies using CRISPR/Cas9 globally and realizing its great potential.

TC: I would point to the quality of scientists as a challenge. There is not a lot of expertise in this area since the field is so new – particularly in manufacturing. High science cannot be limited to just research departments; we need people who will ask manufacturing, regulatory, and quality questions too. In many areas of drug development, there are pre-existing templates, but for genetic medicines, the lack of familiarity amongst people trained in a much more templated, traditional environment, and believing that the previously tried and tested template is going to work each time, could be a recipe for disaster. We need education across the board. We need to educate and inform patients too so they can understand the reality of these therapeutics, and to alleviate their concerns.

WHAT ARE THE DELIVERY CHALLENGES OF GENE EDITED THERAPEUTICS?

LDJ: Enhancing specificity and minimizing off-target effects are among the top priorities in ensuring the safety and reliability of CRISPR therapeutics. We need efficient delivery methods that target specific cell types or tissues. Advances in delivery methods, such as lipid nanoparticles, will enable precise delivery of CRISPR reagents to the targeted cells or tissues. Understanding the long-term effects of CRISPR-mediated therapeutics also continues to be important for clinical translation.

RH: Gene editing can be used in many ways. At my company, we are focused on cell therapies that we manufacture ex vivo – in part, largely because we can readily deliver using electroporation or other technologies to manipulate the cells at very high efficiency and in large batches. There are only so many cells that you can address in that way, such as T cells, hematopoietic stem cells, and a small number of others. To meaningfully turn the hype of in vivo genome editing into reality, we have to figure out how to deliver these reagents with high fidelity to specific organs within the body. I think this is one of the biggest bottlenecks and biggest challenges for the entire genome editing field. Some of our peers in the space have made some very exciting initial efforts and demonstrated great ability to edit cells in the liver. There is a lot that can be done in the liver, but there's also a lot that cannot be done by targeting the liver. I hope that there are a lot of great academic institutions and companies focusing on innovating in this space because I think it's the key to the future of genome editing.

ER: The delivery method used generally depends on the target cells or tissue. Systemic delivery often uses AAV or a nanoparticle formulation. Ex vivo cell therapy can use a variety of delivery modalities, although AAV, RNA, and plasmids are commonly used. Issues related to re-treatment may also complicate this space. Not all genome editing applications may be 'one and done,' although that may be the goal.

Effective delivery and continued safety will remain the biggest challenges for some time. A safety setback could impact the entire industry and because there are so many diverse clinical applications being explored, many independent groups are involved.

Advanced Therapy Solutions

CGT experience where it counts.

Our tailored solutions and dedication to continuous innovation uniquely allow us to help biopharma pioneers successfully bring cell and gene therapies to the people that need them most.

Learn more about Advanced Therapy Solutions

CardinalHealth™

Credit: Shutterstock

Core Topic
Cell & Gene

Small computer, big news. Researchers from Penn State College of Medicine and Huck Institutes of the Life Sciences have developed a protein-based nano-computing agent that can function as a circuit. "We're engineering proteins that directly produce a desired action," said Nikolay Dokholyan, G. Thomas Passananti Professor and Vice Chair for Research in the Department of Pharmacology. "Our protein-based devices or nano-computing agents respond directly to stimuli (inputs) and then produce a desired action (outputs)." The research shows that cell orientation can be controlled by applying the appropriate input signals, a framework that may be useful in tissue engineering and regenerative medicine.

ALS hopes. Cellenkos has dosed the first of six patients in a phase I/Ib study evaluating CK0803 for treatment of amyotrophic lateral sclerosis (ALS). Developed using Cellenkos' proprietary CRANETM technology, CK0803 is a neurotrophic, umbilical cord blood-derived T regulatory cell therapy that targets the central nervous system. "ALS is a devastating disease with no cure, and we believe that CK0803 has the potential to provide a much-needed treatment option for patients. We are enthusiastic to move forward with this trial and to further explore the potential of CK0803 in ALS and other neurodegenerative diseases," says Tara Sadeghi, Chief Operating Officer of Cellenkos.

Lift-off for Elevate. ElevateBio has announced the closing of $401 million Series D financing led by the AyurMaya Capital Management Fund. All proceeds will go towards the development of their cell and gene technology platforms, including an expansion of gene editing systems and demonstration of multiplex base editing that enable the development of a broad array of in vivo and ex vivo therapeutics. "We're emboldened by the pace of advancements to our technology platforms and continue to drive innovation from concept through commercialization and redefine how companies operate, how products are created, and how disease is treated," said David Hallal, Chairman and CEO of ElevateBio.

AstraZeneca strikes again. Quell Therapeutics has entered into a collaboration, exclusive option and license agreement with AstraZeneca to develop, manufacture and commercialize autologous, engineered Treg cell therapies for two autoimmune disease indications (type 1 diabetes and inflammatory bowel disease). Quell will receive $85 million upfront and is eligible to receive over $2 billion for further development and commercialization milestones. "We are extremely pleased to have AstraZeneca on board as our first major partner. This collaboration builds on our pioneering work to develop exquisitely engineered, multi-modular Treg cell therapies for immune disorders."

IN OTHER NEWS

StemCyte receives FDA go ahead for world's first phase II trial using umbilical cord blood cells to treat post-COVID-19 syndrome

Mission Bio has developed new CRISPR-modified solution – Tapestri – to address challenges in genome editing by measuring outcomes at single-cell resolution

Bayer has partnered with Acuitas Therapeutics for lipid nanoparticle gene therapy delivery technology to support in vivo gene editing and protein replacement programs

Abeona Therapeutics announces additional positive phase III VIITAL results for its EB-101 cell therapy trial to treat recessive dystrophic epidermolysis bullosa

Bristol Myers Squibb receives FDA approval for new autologous cell therapy manufacturing facility in Devens, Massachusetts

Power List Perspectives: Challenges Facing Cell and Gene Therapy

Leading pharma industry experts discuss the most pertinent challenges facing cell and gene therapy

Cell and gene therapies are an increasingly proven therapeutic frontier – and look set to play a pivotal role in the future of personalized and precision medicine. To date, more than 25 cell and gene therapies are licensed for use in the US alone, but – as with any evolving innovative approach – researchers and developers face multidimensional hurdles on the road to approval.

The Medicine Maker Power List 2023 (available at https://themedicinemaker.com/awards/power-list/2023) showcases inspirational individuals across the pharma industry – including those from the cell and gene arena. We asked our Power Listers about the most significant challenges standing in the way of progress in the advanced medicine space.

David Backer
CEO, Curate Biosciences
"The use of the technology – and, more importantly, the manufacturing limitations on scale and cost – have relegated cell and gene therapies primarily to diseases that are rare, ultra-rare, or use the body's own immune cells to fight cancer. These are incredible – but fairly localized – successes, and we are still a long way from having cell and gene therapies that are a standard part of therapeutic regimens."

Alan Boyd
CEO, Boyds
"The biggest challenge affecting the field of gene therapy is the manufacturing of the product… When I begin working with a client on advanced therapy, I tell them from the start that they will have issues with their potency assay and other aspects of the product, such as purity. The client must prepare for this eventuality – and bring in the right skills to help."

Tirtha Chakraborty
Chief Scientific Officer, Vor Biopharma
"The issue is ugly science – frequently practiced by our industry. This industry has become so much about the bottom line that we do not appreciate the culture of doing it right. The reward is for getting to the finish line, so most of the bottom line focus is understandable when it comes from the investor community. But the R&D leadership and the management of biotech companies must hold their own, and message their concerns and visions appropriately to the broader community."

Queenie Jang
CEO, International Society for Cell and Gene Therapy
"Workforce development continues to be one of the most significant challenges facing the cell and gene therapy sector. The field has seen exponential growth, which has outpaced the rate at which new professionals enter. On a positive note, we're seeing many newcomers enter the field, but we still have a long way to go to bridge the gap at mid-to-senior levels."

Catherine Jomary
ATMP Lead, IPS-Integrated Project-Services
"The biggest challenges for these new genome editing therapeutics are the specificity of delivery, control of their activity, detection of potential off-target mutations, and their inherent immunogenicity. The goal of an efficient gene editing therapy is to show perfect specificity for the target sequence without mutations introduced to any other region of the genome. Unfortunately, the existing genome editors' systems rarely achieve such a high standard."

Sheila Mikhail
Co-founder, Asklepios BioPharmaceutical (AskBio)
"Like most of the biotech industry, cell and gene therapy companies are facing an investor funding shortage and difficult stock market conditions. Gene therapy continues to work on issues pertaining to the management of the immune system, such as redosing and durability issues. The field continues to make advances to enable more streamlined and cost-efficient manufacturing solutions."

Dirk Nagorsen
Chief Medical Officer, Affini-T Therapeutics
"There are those general challenges with cell therapy approaches for conditions beyond blood cancers, notably finding ways to develop strategies that improve T-cell persistence and durability. Fortunately, we are seeing approaches that aim to address these challenges by leveraging advancements made in computational biology, cellular engineering, gene editing, synthetic biology, and more to enhance T-cell fitness and promote a durable response."

Angela Osborne
CEO, eXmoor Pharma
"It is now well recognized that the biggest challenges of the field are in CMC and in manufacturing in particular. You have to be able to make the products at scale and at a reasonable cost for the cell and gene therapy industry to become as large as the biologics industry is today."

Victor Vinci
VP, Global Product Development, Cell, Gene and Protein therapies, Catalent
"Science is moving so fast with respect to technology innovation and new applications that it creates challenges in establishing the tools to develop, manufacture, and scale up these therapies for clinical trials and potential commercial launch."

Angela Osborne


David Backer


Queenie Jang


Alan Boyd


Victor Vinci


Dirk Nagorsen

DELL Technologies

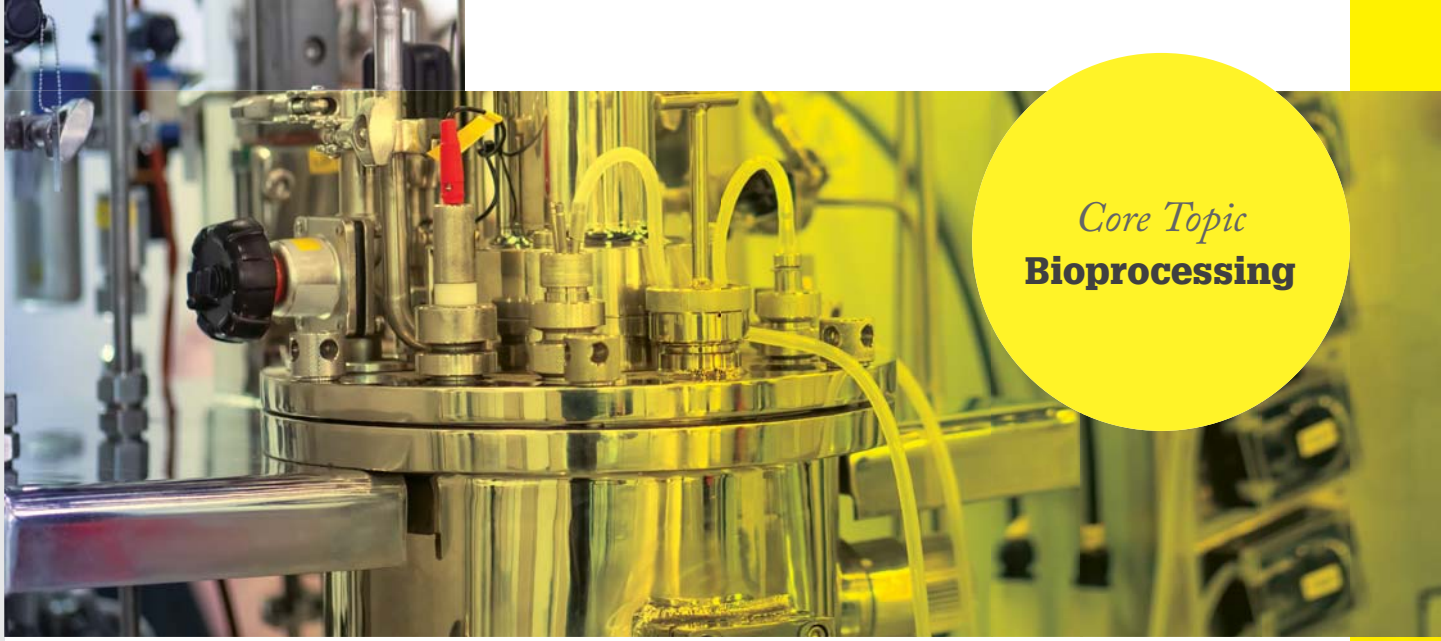
NVIDIA

Accelerating Your Next Discovery

Bring your research to the next level with Dell Technologies & NVIDIA

Learn how Dell's end-to-end solutions with NVIDIA GPU-accelerated compute has greatly improved genomic analysis.

www.Dell.com/Healthcare



Credit: Shutterstock

Core Topic
Bioprocessing

You win some, you lose some. Eli Lilly has received bad news and good news. The company received a complete response letter from the FDA for mirikizumab. The regulator had no complaints about the clinical data package or safety of the medicine for the treatment of ulcerative colitis, but cited issues around manufacturing. In good news, Lilly reports that donanemab met its primary and secondary endpoints in a phase III trial, slowing cognitive and functional decline in people with early symptomatic Alzheimer's disease.

AI proteins. Here's some fascinating research from academia. Researchers from the University of Toronto have developed an AI system called ProteinSGM that can create proteins outside of nature that can still fold into configurations and carry out specific functions in cells. Philip M. Kim, a professor in the Donnelly Centre for Cellular and Biomolecular Research at the university said, "Our model learns from image representations to generate fully new proteins, at a very high rate. All our proteins appear to be biophysically real, meaning they fold into configurations that enable them to carry out specific functions within cells."

Celebrating a career. Regeneron board chair Roy Vagelos retired in early June. He has served as chair since January 1995. In replacement, Leonard S. Schleifer and George D. Yancopoulos will be appointed as co-chairs. Both had warm words to say about their predecessor. Schleifer said, "During Roy's career, he has not only performed at the highest level imaginable, he has raised up all who have had the privilege of working with him." Yancopoulos added, "P. Roy Vagelos has served as my role model since the day in 1975 when my father showed me an article in the Greek newspaper about his similar background and incredible achievements at Merck."

Cytiva update. It's been a busy few weeks for Cytiva. The company has announced the completed integration of Pall Life Sciences, with Pall's biotech portfolio now sitting as a product family in Cytiva's bioprocess business. Cytiva has also launched its X-platform bioreactors in 50 and 200 L sizes. The tech is based on the company's Xcellerex heritage and includes Figurate automation software. The bioreactors also work with the Cytiva Bioreactor Scaler to determine the optimal target settings for scaling without trial and error. In addition, the company has worked with Australian CDMO BioCina to expand a facility in Adelaide to manufacture mRNA-based vaccines and therapies.

IN OTHER NEWS

FDA eases (but does not completely lift) the partial clinical hold on Avidity Biosciences' phase I/II trial of AOC 1001 to allow more participants to be recruited

Aurigene Pharmaceutical Services to invest $40 million in R&D and pilot scale facility for proteins, antibodies, and viral vectors

Sartorius and va-r-tec form partnership to develop safer and simpler transport systems for biopharmaceuticals using single-use solutions and thermal insulated containers

FDA approves Epkinly bispecific antibody for patients with relapsed or refractory diffuse large B-cell lymphoma; drug to be co-commercialized by Genmab and Abbvie

MSD agrees to acquire Prometheus for $200 per share, which values the transaction at around $10.8 billion

The Imperfect Art of Biosimilars

Need a refresher on biosimilars? Here, we offer some key points to keep in mind when it comes to structure, function, and interchangeability.

By Dave Li, KCR Principal Consultant and Anna Baran, KCR Chief Medical Officer

A biosimilar, by definition, is a biologic that is highly similar – in structure and function(s) – to a registered reference biologic, with no clinically meaningful differences in terms of safety, purity, and potency. Biosimilars are regulated in a somewhat different context compared with chemically derived small molecule generics. Regulatory agencies mandate that biosimilars be exchangeable in clinical practice with a reference product. However, in practice, exchangeability has a specific definition that can be vague in meaning. Here, we discuss the concept of interchangeability for biosimilars and offer some operational perspectives on interchangeability considerations in the design and execution of preclinical developments leading to later phases of clinical studies on biosimilar products.

The structural basics

Biologics as functional biosimilars require corresponding structures. Many marketed biosimilars are, of course, proteins – monoclonal antibodies, enzymes, and so on. And a distinctive feature for functional biologics as proteins is correct folding into a tertiary (3D structure) after translation with the appropriate configurations of post-translational modifications (PTMs), such as glycosylation, ubiquitination, and methylation (1). In the cellular networks, there are more than 400 different types of PTMs that can impact many aspects of protein functions (2). Appropriate PTMs ensure correct folding and, therefore, how effectively surface receptors and/or other allosteric binding sites are exposed.

The scientific principles underlying the quality of a biosimilar mandate careful attention to PTM patterns to ensure correct protein folding for functionality – and that means the manufacturing processes must be well controlled (3, 4).

From a safety point of view, the biosimilar and reference product should have similar antigenicity and in vivo toxicity profiles; however, ultimately, adverse events in humans will not be known until first-in-human data for the biosimilar become available. Regardless, reliable in vitro structural and functional data are helpful in planning next development steps, including study designs and executions

The current systems of protein expression for industrial-scale manufacturing are based on imperfect science, which makes the manufacturing process for biosimilars as much an art as a solid science. In particular, there is a lack of understanding when it comes to the underlying cellular and molecular mechanisms of action for PTMs because these are enzyme-mediated and highly dynamic processes.

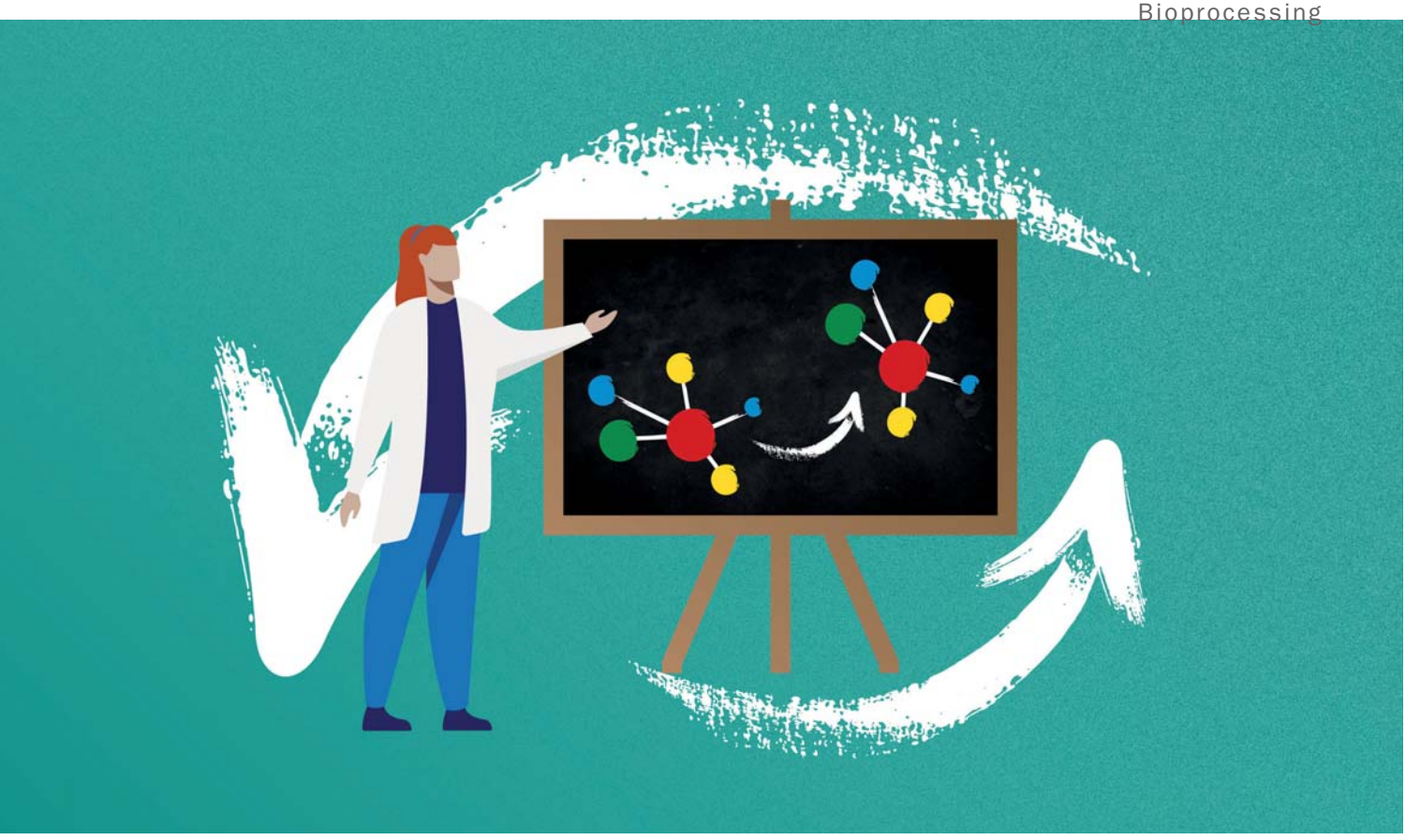
Folding patterns also determine correlation to other biological characteristics, such as toxicity profiles. Unlike small molecule generics, biological drugs need to interact with the tissue and cells of human systems by binding to cell receptors to elicit a downstream therapeutic effect. This is fundamentally different to the classic pharmacodynamic model of absorption, distribution, metabolism, and excretion model. The biological reactions, both in potential side effects and therapeutic responses from biosimilars, are multi-systemic and, therefore, difficult to predict from pre-clinical animal models. As a result, reactions can be highly complex depending on the nature, administered routes, strength, and dosage form (5).

What regulators want

Current EMA and FDA guidelines stipulate that biosimilars should be interchangeable with their reference product, without further intervention from prescribing physicians. The FDA has been clear about the scientific risks and benefits of interchangeability. A biosimilar can be expected to produce the same clinical result as the reference product in any given patient of the target population. And for a product administered to a patient during a treatment course, the risk in terms of safety or diminished efficacy of alternating or switching between use of the biosimilar and its reference product should not be greater. The FDA has developed standards for biosimilars, but these can be subject to different interpretations in clinical development (6-9).

In the US, the traditional approval pathway for a biosimilar is a 351(k)-marketing application, which should include, among other specific considerations, information demonstrating structural biosimilarity and functional interchangeability based on data derived from analytical, animal, and clinical studies demonstrating that the biological product is "highly similar." The pre-clinical studies should include structural and functional analyses. Measuring protein folding is also technically feasible with advanced methodologies.

Animal models are used to determine toxicity, toxicity profiles, and initial



dosing. Clinical studies, including early studies and the assessment of immunogenicity and pharmacokinetics or pharmacodynamics, should lend credible support to demonstrate safety, purity, and potency in one or more appropriate conditions of use for which the reference product is licensed.

Extrapolated indications for biosimilars are a concern if there are no head-to-head comparison data with a reference product.

Reaching consensus

The "no clinically meaningful difference" aspect of biosimilars should be a consensus reached by a collective group of stakeholders. However, from a statistical perspective, data supportive of a null hypothesis in randomized trials with appropriate clinical or patient outcomes suggest that claims of biosimilarity are, at least, highly likely.

Every effort should be made to ensure complete sets of data are collected and used to justify downstream clinical developments for biosimilars and to support and facilitate future marketing applications. With the right pre-clinical analytical data, it can be reasonable to assume that a biologic will be highly similar to its reference product in structure, and therefore safety, purity, and potency, before entering later human trials.

References

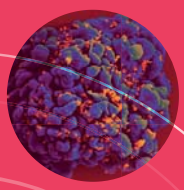
1. *S Ramazi et al., "Posttranslational modifications in proteins: resources, tools and prediction methods," Database, (2021). DOI: 10.1093/database/baab012*
2. *B Atsavapranee et al., "Fundamentals to function: Quantitative and scalable approaches for measuring protein stability," Cell Syst., 16;12, 547-560 (2021). DOI: 10.1016/j.cels.2021.05.009*
3. *P Kurki et al., "Regulatory Evaluation of Biosimilars: Refinement of Principles Based on the Scientific Evidence and Clinical Experience," BioDrugs, 36, 359-371 (2022). DOI: 10.1007/s40259-022-00533-x*
4. *P Declerck et al., "The Language of Biosimilars: Clarification, Definitions, and Regulatory Aspects," Drugs, 77, 671-677 (2017). DOI: 10.1007/s40265-017-0717-1*
5. *SK Niazi, "Biosimilars: A futuristic fast-to-market advice to developers," Expert Opin Biol Ther., 22, 149-155 (2022). DOI: 10.1080/14712598.2022.2020241*
6. *FDA, "Guidance: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product," (2015). Available at https://bit.ly/35mdtJc*
7. *FDA, "Guidance: Considerations in Demonstrating Interchangeability With a Reference Product Guidance for Industry," (2019). Available at https://bit.ly/2VyxVWp*
8. *FDA, "Guidance: Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations Guidance for Industry," (2019). Available at https://bit.ly/2Fhl40n*
9. *FDA, "Guidance: Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product," (2016). Available at https://bit.ly/43IL3aM*

ID Transmission

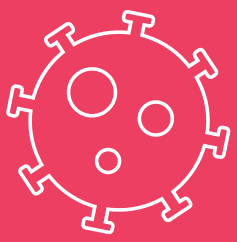
Covering the front lines of the fight against infectious diseases for researchers, clinicians, and policymakers

ID Transmission brings you the latest research and innovations in the field, whilst also tackling hard-hitting and thorny topics that many others shy away from. We keep you informed, start conversations, and connect all disciplines and specialities within the field of infectious diseases.

Visit our website now to find out more
idtransmission.com



Receive updates direct to your inbox by signing up to our weekly newsletter



Join the conversation today

@IDTransmission
ID Transmission
@IDTransmission
ID Transmission

Credit: Shutterstock

Core Topic

Small Molecule Manufacture

Quite quant-rary. AI-driven drug discovery company Insilico Medicine has combined quantum computing and generative AI for drug development, demonstrating the potential advantages of quantum generative adversarial networks in generative chemistry. A study published in JCIM explores hybrid quantum-classical generative adversarial networks (GAN) for small molecule discovery by substituting each element of GAN with a variational quantum circuit, and demonstrating the quantum advantages in small molecule drug discovery (DOI: 10.1021/acs.jcim.3c00562). Insilico scientists now plan to integrate the hybrid quantum GAN model into the company's small molecule generation engine to accelerate and improve AI-driven drug discovery and development processes.

If at first you don't succeed – kill weeds. A molecule developed to treat tuberculosis that failed to progress out of the lab is showing promise as a powerful weed killer, according to scientists at the University of Adelaide, Australia. Lead researcher Tatiana Soares da Costa and her team discovered that by modifying its structure, the molecule became effective at killing annual ryegrass and wild radish, two of the most problematic weeds in Australia, without harming bacterial and human cells. By exploiting the molecular similarities between weeds and bacterial superbugs, the researchers are looking into the discovery of more herbicidal molecules by re-purposing other failed antibiotics.

So far, so good for sotorasib. Memorial Sloan Kettering Cancer Center research demonstrates continued safety and efficacy of sotorasib in patients with KRAS G12C-mutated advanced non-small cell lung cancer. Researchers are now exploring the potential of eradicating tumors by delivering a viral-based immunotherapeutic to melanoma and breast cancer in mice. MSK oncologist Bob T. Li found that sotorasib increased overall survival rate from 14 percent to 33 percent after two years; 23 percent of participants in the trial saw no progression of their disease for a year or more while on sotorasib. The study, sponsored and funded by Amgen, has been published in JCO (DOI: 10.1200/JCO.22.02524).

Valbenazine shows its value. The phase III KINECT-HD clinical trial of valbenazine to treat involuntary muscle contractions, or chorea, in people with Huntington's disease has met all of its primary endpoints, according to Neurocrine Biosciences CMO Eiry Roberts. "There remains a need for symptomatic treatments for chorea associated with Huntington's disease, and this manuscript provides an in-depth overview of the KINECT-HD study data and the potential of valbenazine to fulfill this need," said Roberts. Valbenazine received orphan drug status in 2022, forming the backbone of Neurocrine's FDA application for approval to treat Huntington's-associated chorea. A decision is expected later this year.

IN OTHER NEWS

Freiburg researchers discover mechanism by which cancer cells escape the immune system, and aim to prove efficacy of zolendronate in boosting Gamma delta T cells

Scientists at the Second Hospital of Tianjin Medical University, China, report adverse drug reactions (severe thrombocytopenia) from tirofiban

Swiss Tropical and Public Health Institute researchers test emodepside in human parasitic whipworm infections; results show high efficacy

Researchers in Japan identify compound that can prevent cisplatin-induced renal toxicity and improve cancer treatment outcomes

Scynexis closes exclusive license agreement with GSK for the right to commercialize Brexafemme (ibrexafungerp) tablets

From Plastic Pollution to Pharma Compounds

How fungi help turn ocean trash into pharmaceutical platform molecules

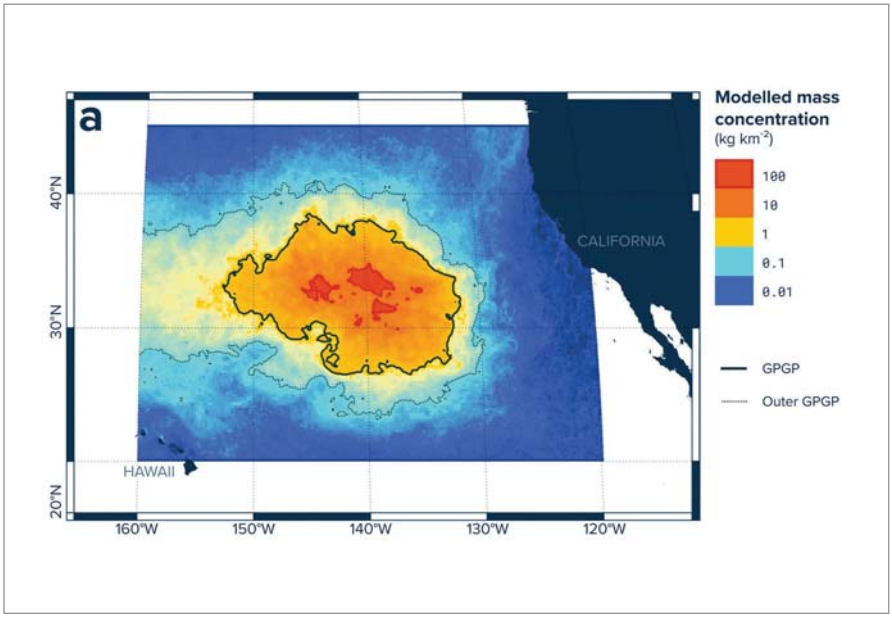
The Great Pacific Garbage Patch is estimated to measure around 1.6 million square kilometers – about three times the size of France – and it consists mainly of plastic.

But one person's trash is another person's treasure, and researchers from the University of Kansas and the University of Southern California have devised an approach that uses genetically engineered soil fungus – Aspergillus nidulans – to convert plastic from the patch into more useful compounds (1).

"At this point we've reported the conversion of polyethylenes, polystyrene, and mixed, rigid, ocean-sourced plastics to several pharmaceutical platform molecules, including asperbenzaldehyde, citreoviridin, mutilin, and pleuromutilin. We've also produced ergothioneine and Af36 spores, the latter used on scale as a biocontrol agent," says Travis Williams, a professor at the Wrigley Institute of Environmental Studies at the University of Southern California, and one of the authors of the paper describing the approach.

The researchers used oxygen and metal catalysts to digest polyethylenes into carboxylic diacids, which were then fed to the Aspergillus fungi to "upgrade" the diacids into "structurally diverse secondary metabolites."

Williams says, "My colleague, Clay Wang, and I are both upset about the Great Pacific Garbage Patch. He found



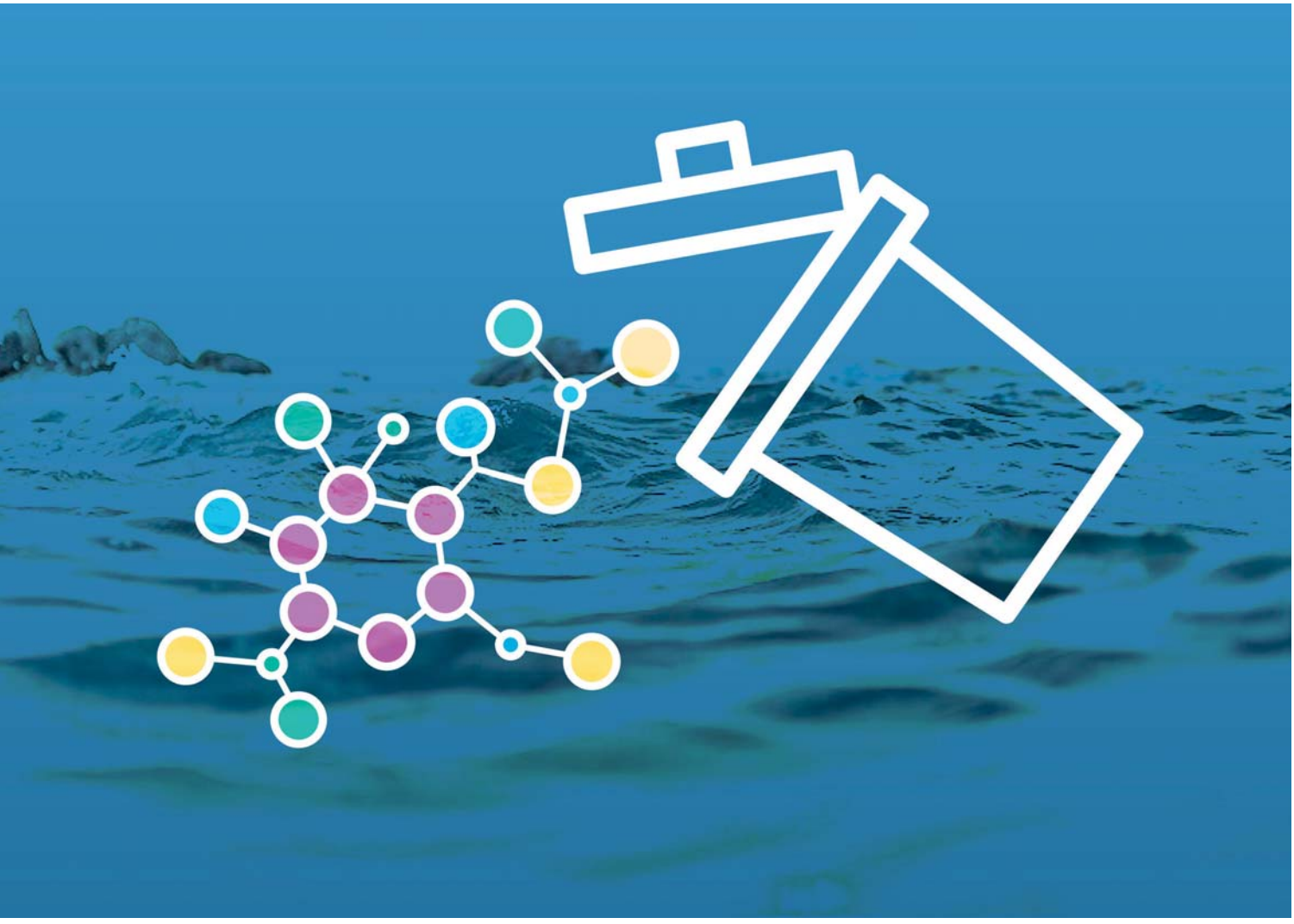
The Great Pacific Garbage Patch in 2017 *Credit: Wikipedia, L. Lebreton, B. Slat, F. Ferrari, B. Sainte-Rose, J. Aitken, R. Marthouse, S. Hajbane, S. Cunsolo, A. Schwarz, A. Levivier, K. Noble, P. Debeljak, H. Maral, R. Schoeneich-Argent, R. Brambini, and J. Reisser*

insight from literature that suggested some fungal strains might recognize carboxylic diacids as metabolic substrates, and that these might be prepared by oxidative cleavage of polyethylene. He asked me if we could work out a chemical process for the polymer cleavage, which my lab was able to do. With some optimization, we worked out the fungal growth conditions based on oxidatively cleaved polyethylenes. We then started testing out the system on mixed plastics and other polymers and found unexpected success."

"With any recycling approach, one significant challenge is ensuring it can cope with real-world plastic."

In fact, researchers at the University of Kansas described the approach as "bizarrely efficient." According to Williams, the team recovered 83 wt% of diacid products from a clean sample of low-density polyethylene (LDPE). "Digest materials from this reaction were converted to asperbenzaldehyde in ca. 40 atom% efficiency. Those numbers are staggering to me, and certainly not all of our experiments are that successful," he adds. "We designed our approach to be tolerant of real-world plastic waste, but the day we had actual fungi growing on materials that we harvested from the garbage patch was the day I knew we had made an important contribution."

With any recycling approach, one significant challenge is ensuring it can cope with real-world plastic. The chemistry developed by the researchers can cleave hydrocarbon polymers – as well as ocean tar, which frequently sticks to the samples. Williams explains that



these digests are generally well-tolerated by the fungal systems. "We're still working through controlled experiments to see which are better tolerated than others. Polyethylenes and polystyrenes work well. Nylons, polyesters, polyurethanes, and related materials are degraded, but we don't know about fungal upgrading yet. We haven't studied the less-common engineering polymers (ABS, PEEK, PES, and so on). We haven't studied PVC or PFASs, but we don't expect them to work. We have applied the chemistry to thermoset epoxies and epoxy composites; it works, but the best products we make do not use biocatalysis.

Williams is hopeful that it will be possible to adapt and scale the process to manage unsorted, unfiltered plastics wastes, such as those found in recycling centers. "I see a great number of hurdles to that goal, but they all seem surmountable to me," he says. "A question that we struggled with for a long time was what relevance this system would have on global plastics management if the largest volume products that we were making were high value/low volume pharmaceuticals. But we can also make agricultural products, so we may be able to make a dent in global waste management. I think there will be a strong business case to scale this technology to a meaningful size."

How else do you think this technology could be adapted? Williams is keen to hear your feedback: travisw@usc.edu. After all, the potential of genetic technology to produce a broad diversity of products is almost endless…

Reference

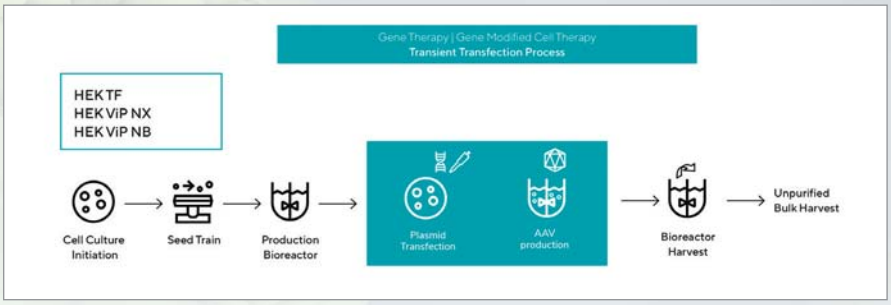
1. *C Rabot et al., Angewandte Chemie (2022). DOI: 10.1002/anie.202214609*

Scaling Gene Therapy Challenges – Together

Understanding AAV production and characterization processes – and establishing strategies for success

By Catherine Buchere, Product Manager Virus Based Therapeutics and Kathrin Teschner, Manager of Viral Vector Technologies, both at Sartorius

Figure 1. Overview of Transient Transfection Using AAV Vectors



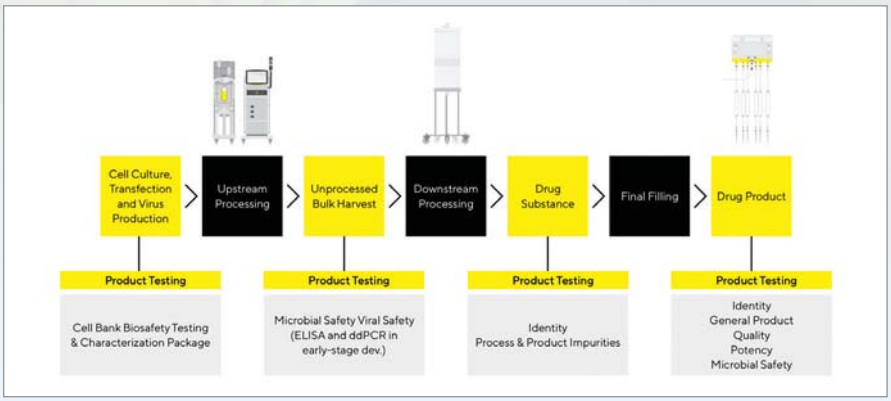


Figure 2. Biosafety and Characterization Testing Methods Are Required at Every Stage of AAV Product Manufacturing

Viral vector gene therapies show great promise for treating a myriad of diseases, and the excitement across the field is palpable. As more therapies approach commercialization – and as interest grows in using gene therapies for broader indications – biomanufacturers will need to establish manufacturing strategies that help them to respond to the anticipated surge in demand. Gone are the days of targeting only rare diseases with resulting low demand – the gene therapies of the future will demand optimization of all aspects of manufacture and characterization.

As you will know, the leading viral vector for gene therapy is the adeno-associated virus (AAV) – a small non enveloped, single-stranded DNA virus with a diameter of 18–25 nm. Thanks to their low immunogenic profile, which complements low pathogenicity during gene delivery and reinforces safety, AAVs are a powerful delivery vehicle. Moreover, different AAV serotypes can exhibit specific tropisms for specific organs and tissues of the body to improve targeting (1) – and new serotypes are continuously being discovered.

Understanding the production challenge

The production of recombinant AAV vectors with high purity and potency – crucially, while maintaining good yield – is complex. Indeed, when it comes to manufacture of a given viral vector, there is no standard and robust approach to upstream and downstream processes.

Most commonly, AAV production employs a three-plasmid transfection model to encode the gene of interest, packaging rep/cap, and helper genes. This transient triple transfection allows for yields in the range of ~10^3–10^5 vector genomes per cell – and the process is well established, being widely adopted in research laboratories. But adapting the process to a bioreactor environment for scale up can present challenges, especially when adherent cell cultures are still used for production.

These scale up challenges have led many process development teams to focus their manufacturing strategy on developing a producer and packaging cell lines, with the components necessary for rAAV production integrated into the genome (either partially or totally). This approach brings scalability benefits and avoids the critical step of transfection, but there are drawbacks – not least the time and upfront process development required to develop such a stable cell line – as well as the financial investment. Importantly, each serotype/vector combination requires the generation of a unique cell line.

It's clear that both approaches have disadvantages as well as advantages – but, right now, they remain the main options for viral vector production. The choice between them typically depends on the timeline, budget, and the gene therapy being developed.

Know your analytical methods!

Experts at Sartorius have produced a poster titled, "Analyzing AAV – A Story of Problems and Solutions" (2). Although AAV has many advantages for gene therapy and has thus gained an outstanding reputation, the efficacy and safety of AAV-based gene therapy is dependent on an optimal manufacturing process followed by robust characterization processes that reveal titers of capsid as well as the vector genome.

Optimizing Through Serum-free Media

Scale up can be challenging and bottlenecks are amplified when using adherent cell lines. One key to the success of the AAV production process upstream is selection of the optimal media for the cell line expression system. To sustain growth and productivity, high-performing media should mimic the production cell's natural environment, such as energy source (glucose), vitamins, amino acids, trace elements, lipids, hormones, and salts.

Currently, two main cell lines are used to produce AAV gene therapies: human embryonic Kidney 293 (HEK 293) and insect cells isolated from the fall armyworm Spodoptera frugiperda (Sf9). One of the approaches to circumvent processing bottlenecks and scalability challenges is to adapt cell lines to suspension cultivation, and then transition to a bioreactor on a large scale (50 L) at the start. Suspension cell processes in serum-free media can be operated in batch or fed-batch mode to enable high yield.

Advantages of using serum-free media include more consistent performance, increased growth and/or productivity, better control over physiological responsiveness, and reduced risk of contamination by serum-borne adventitious agents in cell culture.

Sartorius is a well-known supplier in the biotech world and offers technologies for every phase of the biopharmaceutical value-added chain. Known as a pioneer in single-use bags and filters, Sartorius has massively invested in the science and manufacturing of the cell culture media for gene therapies, protein-based, and advanced cell therapy modalities.

For gene therapy applications, we offer free samples of our HEK media kit. Request yours at www.sartorius.com/en/pr/request-sample-kit-hek293

Various methods are available for vector characterization – but again, we face a conundrum of advantages and disadvantages, which are shown and compared in our poster. ELISA is probably the most used method to determine the total number of capsids in a sample, but the value is often accompanied by a high coefficient of variation (CV). An additional method is needed to determine the proportion of full capsids, and the genomic titer is usually determined by qPCR (although we prefer newer methods, such as ddPCR due to greater robustness). Genomic and capsid titers divided subsequently give the full:empty ratio – but this result is, of course, affected by the combined error of the two methods.

Another analytical technique that can be used for characterization is size-exclusion chromatography with multi-angle light scattering detection (SEC-MALS). This approach allows determination of several AAV quality attributes in a single measurement, including total capsids, full capsids, and aggregation. The disadvantages? In addition to the lack of high throughput, the sample must be purified beforehand, which makes in-process control more difficult. Additionally, user-related deviations must be considered – as must the measurement error of the method.

Is there a third option to consider? Determination of the genome and capsid titer using parallel purification of the sample is also possible using an affinity chromatography method we developed in-house (outlined in our poster). It has high precision and compares well with the results of the other methods. But it too has a drawback: When titers are low, the sample volume needed for reliable analysis is high…

It's also worth remembering that, depending on the development phase of the gene therapy, analytical priorities may vary. For example, during process optimization, the need for in-process control and high throughput is high. But to ensure important quality attributes in the in vivo gene therapy, other factors, such as precision and robustness, become the highest priorities and thus decisively determine which methodology should be chosen.

So, what is the overall message of our poster? Know your methods! All methods for determining AAV quality attributes have advantages and disadvantages. Without an existing certified reference, each method can be optimized in terms of precision – but which method ultimately represents the true titer? Right now, the industry doesn't have all the answers. But by combining different measurement methods perhaps we have a chance of getting as close as possible to the true value from a large data set.

Scaling the mountain together

The process of understanding viral vector production is a mountain, where complete knowledge represents the summit. Despite years of research in the field – and especially the focus on AAV in recent years – the industry is not yet at the summit, but we are inching closer. In fact, the scientific community has been climbing for years and we have made it through some particularly difficult terrain, but we must keep climbing.

Notably, the biggest mountains are not conquered alone – it almost always takes a team. To understand viral vector production – and more specifically AAV production – we need to make a concerted effort to combine methods and deploy expertise from different fields. And that's exactly the approach we have tried to take at Sartorius. With our poster, we wanted to show how customers can combine different methods to gain even more knowledge – and to keep everyone moving in the right direction. One day, we will reach that summit – together.

References

1. *S S Issa et al., "Various AAV Serotypes and Their Applications in Gene Therapy: An Overview," Cells, 12 (2023). DOI: 10.3390/cells12050785*
2. *Sartorius, "Analyzing AAV – A Story of Problems and Solutions" (2022). Available at https://bit.ly/3o6axhU*

NextGen

R&D pipeline
New technology
Future trends

mAbs: Not So Sweet

How fucosylation-deficient CHO cell hosts can help enhance the potency of monoclonal antibody-based biotherapeutics

By Neha Mishra, Senior Scientist Bioproduction and Jesus Zurdo, Global Head Cell, Gene Therapy & Bioproduction at PerkinElmer's Horizon Discovery

Early on, one of the central challenges in mAb production was low product titer – but this has since been overcome by advances in cell line development and substantial improvements in cell-specific productivity, and further driven by broader progress in industrial bioproduction technology. Bioprocess optimization, generally achieved via optimization of media and culturing conditions (temperature, speed, etc.), has led to significant improvements in product titers and performance of the host cells. Meanwhile, the ever-increasing demand to develop and manufacture mAbs has led to heavy investment in R&D programs focusing on product quality and consistency.

Approval of biotherapeutics for human use requires the definition and control of a number of critical quality attributes (CQAs) which are key to performance and safety. For mAbs, the presence and type of post-translational modifications (PTMs), such as glycosylation, is a good example (2). Glycosylation is an enzymatic process involving the addition of oligosaccharide structures to specific amino acid sites of polypeptides to form glycoproteins. This non-template based process occurs within the endoplasmic reticulum (ER) and Golgi as the protein transits through the cell before secretion or translocation. There are many forms of glycosylation, but the two most common types are N- and O-linked glycosylation:

- In N-linked glycosylation, oligosaccharides are attached to the amide nitrogen of an asparagine (Asn) residue in a consensus sequence Asn-X-Ser/Thr where X is any amino acid except proline.
- In O-linked glycosylation, oligosaccharides are attached to the oxygen atom of hydroxyl groups of amino acids such as serine (Ser), threonine (Thr) or tyrosine (Tyr).

The glycan core structure (see Figure 1) presented by antibodies contains N-acetylglucosamine (GlcNAc) and mannose upon which other sugar residues, such as galactose, sialic acid and fucose, are added.

Why is glycosylation so important in proteins?

Approximately 70 percent of mammalian proteins are glycoproteins with N-linked glycans, which often confer specific properties to the polypeptide chain. Variation in N-glycosylation of therapeutics can have a significant impact in protein folding, stability, pharmacokinetics, immunogenicity, or even mode of action (2, 3). This impact is particularly relevant for mAbs, where variability in the N-glycan structures present in the CH2 domain determines, amongst other things, cell-mediated responses, including antibody-dependent cell cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC).

Given the influence of specific glycans on the therapeutic effect of biologics, the control of glycosylation profiles in biopharmaceuticals, is a highly important topic.

ADCC responses are mediated by the FcγRIIIa (CD16) receptor expressed primarily by natural killer (NK) cells (also known as effector cells). Antibodies recognizing specific ligands on a "target-cell" surface can activate NK cells through the interaction between the Fc region of the antibody and the FcγRIIIa receptor of an NK cell, resulting in release of cytotoxic agents that ultimately eliminate the target cell (see Figure 2). The magnitude of the ADCC response is dependent on the affinity between the FcγRIIIa receptor and antibodies (4). Structural studies have revealed that the presence of fucose on the core glycan structure on IgG1-Fc reduces binding affinity of the IgG1 to FcγRIIIa receptors (5). Therefore, the removal of core fucose in glycan structures of antibodies – known as afucoslylation – is a particularly important strategy in oncology therapeutics.

Figure 1. Glycan core structure

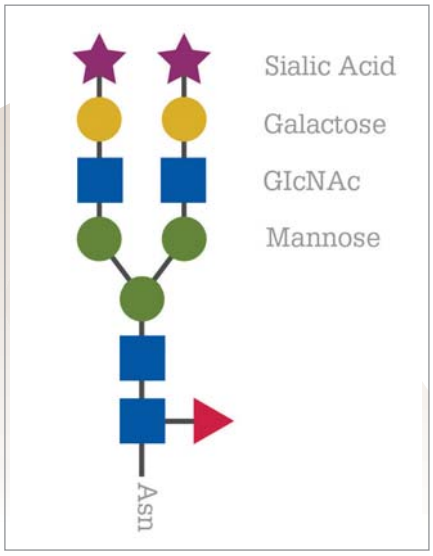
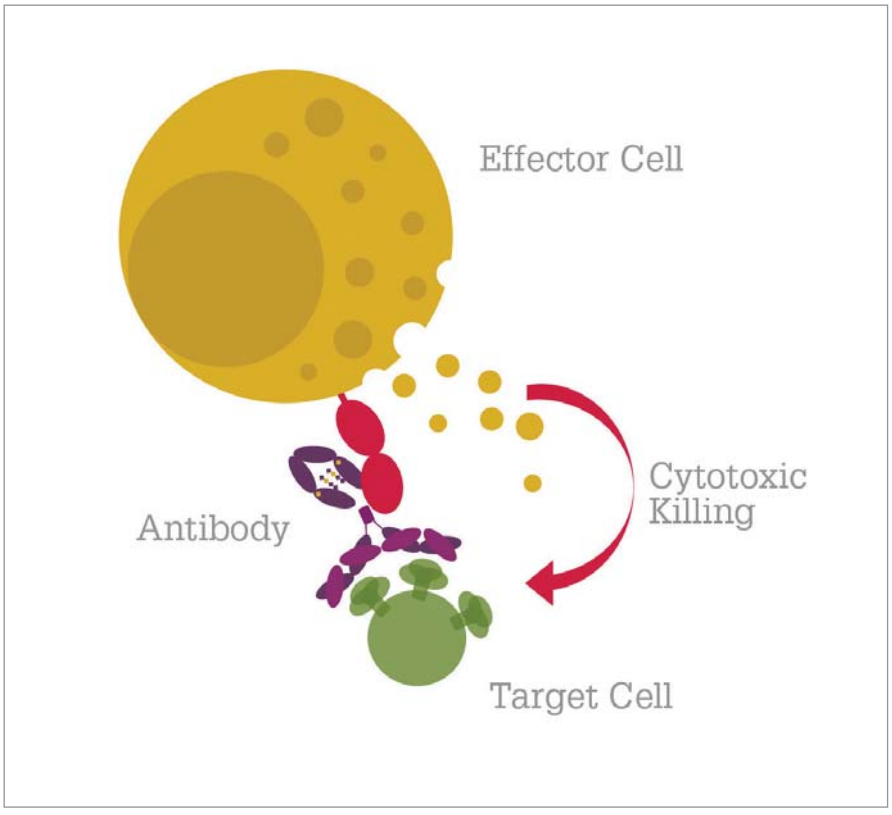


Figure 2. ADCC mediated by effector cells: ADCC response on a target cell via CD16 receptor on an effector cell, triggered with the help of a mAb (figure adapted).



Advantages of afucosylated antibodies include:

- Effective ADCC responses against tumors exhibiting low antigen-expressing levels. This can be relevant for cancer therapeutics, such as Rituximab, which has been shown to be less effective against lymphomas with reduced CD20 expression (6). The ability of afucosylated mAbs to elicit ADCC responses against cells with low antigen expression levels opens the door to more effective therapeutic approaches against currently unsuitable oncology targets.
- Reduced competition from serum IgGs in binding (and activating) FcγRIIIa receptors. Evidence in clinical settings has shown that therapeutic antibodies can be inhibited by circulating IgG competing for FcγRIIIa receptor binding (7). Higher levels of therapeutic antibodies are therefore required to overcome this competition, which can introduce complications and undesirable side-effects. The use of afucosylated antibodies can reduce such competition by increasing the binding affinity to FcγRIIIa receptors.

By addressing these two factors, afucosylated antibodies could have a significant impact in increasing the potency of biopharmaceuticals, expanding their therapeutic window, and potentially reducing undesirable side-effects and complications associated with treatment, due to the lower doses required to elicit a physiological effect.

The use of glycoengineered mAbs is not restricted to oncology therapies. Complement-dependent cytotoxicity (CDC) is also affected by the glycosylation pattern; antibodies exhibiting low or no galactose and high mannose show a decreased binding to complement component 1q (C1q) complex, leading to a reduced CDC response. Additionally, highly sialylated antibodies can mediate anti-inflammatory responses in autoimmune diseases (8).

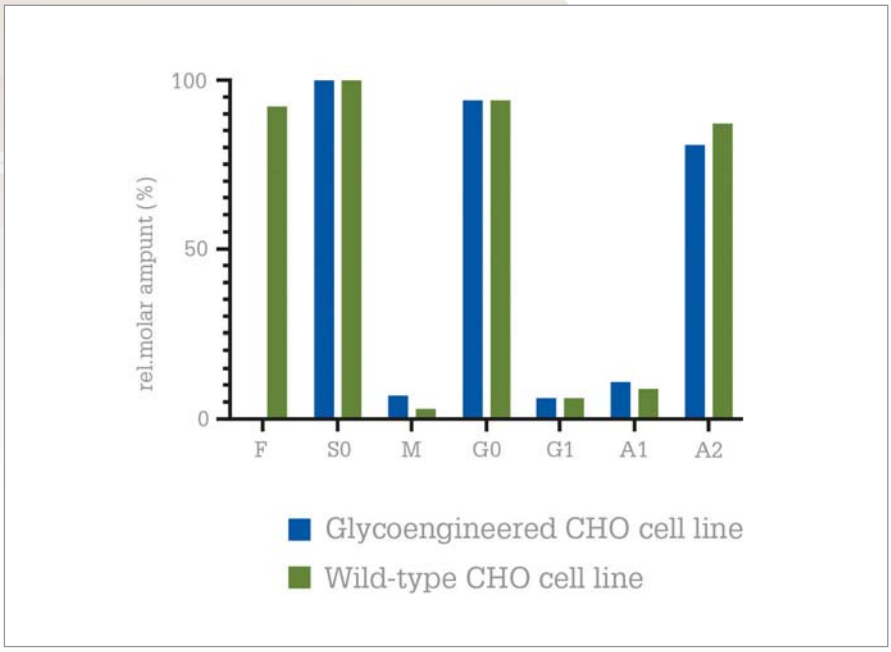
Given the importance of glycosylation on effector functions that are mediated by therapeutic antibodies and Fc-fusion biotherapeutics, host cell lines used to express such products can be engineered to produce selective glycoforms that can, in turn, modulate their specific biological activity.

The right tool for the job…

Chinese hamster ovary (CHO) cells have been used for biologics production since the approval of t-PA in 1987. CHO cells can produce human-like PTMs and are robust systems capable of adapting efficiently to different culture conditions, including serum-free media. Importantly, CHO cells are less prone to being infected by human viruses. Recent advances in bioprocess engineering have dramatically increased the performance of these cells and the yields typically obtained in bioproduction (9).

CHO cells usually produce high proportions of fucosylated mAbs,

Figure 3. N-Glycan structures detected on trastuzumab (TTZ) control mAb expressed in a wild- type and glycoengineered CHO cell line detected by HILIC-HPLC. TTZ produced in the glycoengineered cell line shows complete removal of fucose from the glycoprotein (data from PerkinElmer's Horizon Discovery).



impacting the biological activity of antibody therapeutics they express. Equally, as stated above, other glycan modifications can drastically influence the effector function of mAbs. Therefore, there is great potential in the modification of the glycosylation pathways of CHO cells to generate therapeutics with improved properties. For this, the use of next-generation genome editing tools can offer an effective tool to engineer expression hosts able to produce therapeutics with specific characteristics (10).

There has been a growing interest in controlling the glycan composition of therapeutic proteins, particularly to generate more efficacious therapies by eliminating the fucose content of mAbs. To enrich the proportion of afucosylated antibodies in the final product, several strategies have been explored: (i) control of cell host (CHO primarily) metabolism during cell culture conditions, (ii) inhibitors targeting fucosyltransferase or other fucosylation enzymes, (iii) expression of enzymes to deviate metabolism, reducing available fucose in the cells, and (iv) use of RNAi to repress or reduce transcription of key fucosylation enzymes, amongst others.

However, glycan composition is highly sensitive to external conditions, product, and overall behaviour of cells in culture. Consequently, this creates a problem for developers on two fronts: i) most of these technologies make it virtually impossible to generate therapeutic preparations with 0 percent or 100 percent of their molecules containing a given glycan composition (8), and ii) batch-to-batch variability observed in bioproduction is intrinsically inherent to the nature of the cell culture control systems – and can have significant consequences in drug potency and safety. The latter is particularly acute because potency cannot be simply traced to dose anymore and batch-to-batch variations in glycan composition can have a substantial impact in drug potency. This places additional stresses on manufacturing and quality control that are very difficult to address.

Therefore, there is great potential in the modification of the glycosylation pathways of CHO cells to generate therapeutics with improved properties. In this regard, next-generation genome editing tools can help engineer expression hosts able to produce therapeutics with specific characteristics (10).

Two glycoengineered mAbs lacking fucose, anti-CCR4 mogamulizumab and anti-CD20 obinutuzumab, have been approved for therapeutic use in 2012 and 2013, respectively (both produced in genetically modified CHO cells). Many more glycoengineered mAbs lacking fucosylation are currently in development in areas as diverse as oncology and infectious diseases (8).

When it comes to fucose, one obvious answer lies in engineering host variants that lack the ability to incorporate a fucose molecule in the glycan structure (11). In these types of systems, it is possible to use a functional knockout of a fucosyltransferase gene to inactivate the fucosylation pathway in the cells. Antibodies expressed from these cell lines contain glycans that are devoid of the core fucose as shown by glycan analysis, where 0 percent of fucose is detected. In comparison, mAbs produced from the wild-type parental cell line contain up to around 90 percent of fucosylated glycans (see Figure 3). Afucosylated model antibodies exhibit markedly higher efficacy in eliciting an ADCC response than their fucosylated counterparts when faced with target cells with low antigen-expressing cells and in the presence of NK cells with FcγRIIIa receptor polymorphisms that are known to decrease ADCC functionality (12).

Understanding glycobiology

As outlined above, glycan composition is well known to modulate the biological activities of antibodies in our bodies – from regulating half-life to eliciting ADCC or CDC immune responses. Typically, these functions are mediated via endogenous Fc receptors present in different cell types and tissues and influenced by their relative

affinity for different Fc architectures (including different amino-acid and sugar compositions).

Glycobiology is, therefore, emerging as an important discipline in the design of more effective biotherapeutics, particularly by modulating effector function in the case of IgG molecules. As we've also highlighted, gene editing technologies can be used to engineer host cell lines able to produce afucosylated therapeutic antibodies to enhance ADCC response; indeed, antibodies lacking fucose in their Fc glycan show up to 50-fold increased binding affinity to FcγIIIa receptors of NK cells mediating effector ADCC responses (12, 13). The absence of fucose residue also compensates for the differences in effector function activities across human populations with different polymorphisms in position 158 of the FcγIIIa receptor. More broadly, afucosylated antibodies have shown improved patient responses and outcomes, irrespective of the amino acid present at such a position (13). And this adaptive immune response has much wider applications beyond the development of treatments for oncology, opening the door to applications in a wide range of conditions where better control over ADCC effector function activity is desirable.

The development of antibodies with enhanced ADCC activity has also been increasingly explored in the treatment of infectious diseases, particularly viral infections; there is a growing body of evidence supporting the use of cytotoxic mechanisms of action to control the spread of infection within patients affected by a given virus. This approach has been successfully assessed against a number of different infections, including Ebola virus, human immunodeficiency virus (HIV), respiratory syncytial virus (RSV), and influenza (14).

In short, genetically modified CHO cells can be used to produce afucosylated antibodies with enhanced ADCC activity, which can drive the development of more effective treatments in oncology, infectious diseases, and autoimmune disorders, while offering greater control over product quality and potency.

References

1. *G Walsh, Nat. Biotechnol., 36, 1136–1145 (2018). DOI: 10.1038/nbt.4305*
2. *D Reusch, ML Tejada, Glycobiology, 25, 1325–1334 (2015). DOI: 10.1093/glycob/cwv065*
3. *DN Hebert, SC Garman, M Molinari, Trends Cell Biol., 15, 364–370 (2005). DOI: 10.1016/j.tcb.2005.05.007*
4. *UJE Seidel, P Schlegel, P Lang, Front. Immunol., 4, 1–8 (2013). DOI: 10.3389/fimmu.2013.00076*
5. *C Ferrara et al., Proc. Natl. Acad. Sci. U. S. A., 108, 12669–12674 (2011). DOI: 10.1073/pnas.1108455108*
6. *MR Smith, Oncogene, 22, 7359–7368 (2003). DOI: 10.1038/sj.onc.1206939*
7. *S Preithner et al., G. Mol. Immunol., 43, 1183–1193 (2006). DOI: 10.1016/j.molimm.2005.07.010*
8. *R Mastrangeli, W Palinsky, H Bierau et al., Glycobiology, 29, 199–210 (2019). DOI: 10.1093/glycob/cwy092*
9. *KP Jayapal et al., Chem. Eng. Prog., 103, 40–47 (2007).*
10. *J Zurdo, A Gough, D Cougot, IBI, 3, 44–47 (2020).*
11. *N Yamane-Ohnuki et al., Biotechnol. Bioeng., 87, 614–622 (2004). DOI: 10.1002/bit.20151*
12. *N Yamane-Ohnuki, M Satoh, MAbs, 1, 230–236 (2009). DOI: 10.4161/mabs.1.3.8328*
13. *SD Liu et al., Cancer Immunol. Res., 3, 173–183 (2015). DOI: 10.1158/2326-6066.CIR-14-0125*
14. *G Salazar et al., npj Vaccines, 2, 19 (2017). DOI: 10.1038/s41541-017-0019-3*

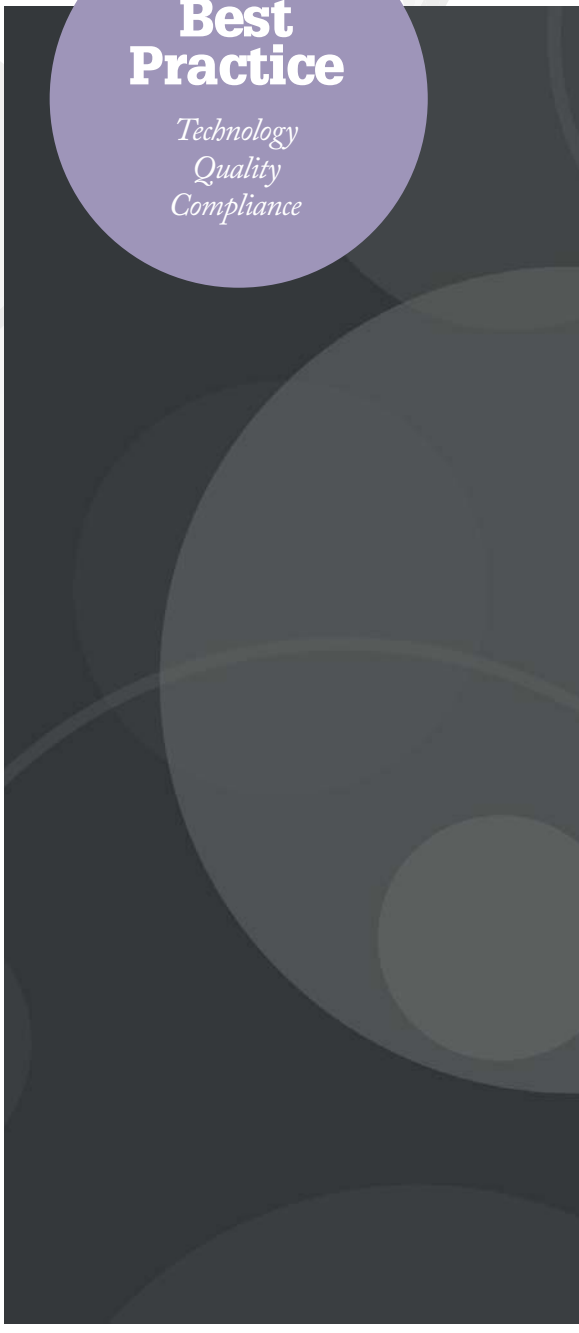
Best Practice

Technology
Quality
Compliance

The Dark Crystal

Though crystallization is considered a "black art" in some circles, it's still a crucial part of the API manufacturing process

By Andrew Blythe-Dickens, Solid State Scientist at Sterling Pharma Solutions



Crystallization may not be a new technique for isolating molecules, but it remains a critical part of the manufacturing process for many APIs. The technique is both effective in purifying the material and producing desired attributes for further downstream processing, including drug product formulation.

Crystallization should be seen as more than simply isolating a compound; it is an excellent way of improving quality at any stage; especially given that impurities can cause problems with subsequent onward processing. Controlled crystallization offers the opportunity to control both the polymorph and the particle morphology (the shape and size) of the resulting crystals.

Controlling particle size is most important in the final step of API manufacturing from a specification point of view. However, it is still important to consider physical properties in earlier stages of the synthesis because size can detrimentally impact processability of subsequent steps; for example, very fine particles may be difficult to filter efficiently, while suspensions of large particles may be difficult to line transfer between vessels during production.

"Crystallization should be seen as more than simply isolating a compound; it is an excellent way of improving quality at any stage."

As with particle size, controlling polymorphs is most important for the final step, but may also have implications earlier on; for instance, high levels of residual solvents or water may cause the formation of a solvate or hydrate form of an intermediate molecule that might interfere with the next chemical step.

The crystallization process

Various solid form chemistry studies are required to inform the development of an effective crystallization process. As well as a solvent screen and solubility evaluation, it is important to carry out a polymorphism study to identify whether there are any different crystal forms that might be made during crystallization, depending on the conditions. If the crystallization is of an intermediate, this study need not be too intense. However, if it is the final API, it will need to be more comprehensive because of the implications for the formulation of the drug product.

For a successful crystallization, the molecule typically needs to be soluble in one or more of the standard process solvents and be chemically robust. Other output requirements for an API, such as particle size distribution, will very much depend on what is required for a successful drug product formulation.

If a successful crystallization and suitable crystal form can be found, this may remove the need for a range

of additional work. Of course, if the material is an intermediate, only solubility, purity, and some chemical stability data will be required to aid the design of the process. If the API falls into Class 1 or 2 on the Biopharmaceutical Classification System (BCS), where solubility of the drug substance is less of an issue, then a comprehensive preformulation evaluation may not be required. However, if it falls into Class 3 or 4 with low solubility or permeability, particle size control may be essential to achieve efficacy.

On some occasions, a suitable crystal form may not be achievable. Investigation into an alternative stable polymorph may be an option, which could offer alternative habits that might be suitable instead. If the molecule has one or more ionizable moieties in its structure, it is very possible that a salt version of the material could be successful; whereas if the molecule is neutral, then an alternative would be to create a cocrystal, where the API forms crystals that also incorporate a second biocompatible molecule.

Be the early bird

There are often significant advantages in determining a suitable crystallization early in the development process. Taking this approach will involve early collection of data on the material, which can be used throughout development and scale-up. With this work starting earlier – and being conducted in parallel to chemical development – it should be possible to reduce the overall development timeline. If the decision is taken to wait for "process typical" material to be manufactured instead, this work will inevitably become lengthier.

Development priorities may sometimes dictate that speed of material delivery is paramount, which can cause delays in crystallization development.

"In an ideal world, development chemists will take a "right first time" approach and work alongside plant engineers, solid state chemists, formulation experts, and commercial teams to determine the optimal plan to achieve the desired results."

In this instance, if data were collected on an ongoing basis to assist the chemical development team, it can help overcome issues and identify suitable solvents for reactions and clean-out processes. A poorly optimized process may be tolerated on a small scale, but the cost of inefficiency increases exponentially as the process is scaled up.

If the synthetic route needs to be modified, some of the crystallization development work might have to be repeated. For example, the impurity profile of the modified process may have changed and become more challenging to control or perhaps the crystallization performance has been affected by the nature and level of residual solvents.

Equally, the original isolation and crystallization process may remain appropriate – and a performance assessment will inform the decision on whether it is still effective at removing different impurities. The platform to build upon may already be there; for example, solubility data may have already been collected for alternative suitable crystallizations that perform adequately.

In addition to solvent choice, various other factors need to be considered during crystallization studies, including the equipment available. For example, a process may have been developed with vessel configurations that differ in capacity, dimensions, and agitator, and will certainly differ to those available at plant-scale. Modeling and simulation tools are available to aid in designing or translating a process to the appropriate scale that is required – and to ensure that the process still achieves the desired outcome.

Fundamentally, crystallization should always be seeded to achieve the greatest control. Adding an aliquot of seed crystals at an appropriate point will help control the final polymorph and avoid uncontrolled nucleation. The seed point is determined by solubility data and the metastable zone width curve, which is obtained via unseeded crystallization induction experiments. Importantly, the particle size of the crystals can be determined by the seeding process, which can affect whether the API meets any final particle size specification. Seeding comes with its own set of parameters to allow the efficient growth and control of crystals, including its loading concentration, the temperature it is carried out at, the shape and size of the crystal, as well as the seed surface area.

If there is only limited material available to develop the optimal

crystallization, then in silico screening tools (such as COSMO-RS) can be used to predict outcomes. Automated screening instruments can also reduce the amount of material and operator time required in physical studies.

In an ideal world, development chemists will take a "right first time" approach and work alongside plant engineers, solid state chemists, formulation experts, and commercial teams to determine the optimal plan to achieve the desired results.

Alternatives and keys to success

Crystallization is not the only way in which the solid form of an API (or intermediate) can be optimized. Jet milling or micronization are other efficient ways of creating smaller particles of a homogeneous size within parameters that are suitable for making effective solid dose forms, particularly if these smaller particles improve dissolution.

Milling can have other benefits because it could remove the need to develop a crystallization process. Some form of milling is frequently the most effective way of achieving the necessary particle size, particularly if a size of 20 µm (or even lower) is required. However, a milling process will still require development and, unlike crystallization, cannot improve a product's purity. Control of the final polymorph formation may also be lacking – and performance can be affected if there is variable input into the milling process.

When making and isolating intermediates, an alternative to a crystallization might be to telescope the process directly into the next stage without actually isolating the material. This may save time by removing the processing or crystallization step, and reducing the number of inter-batch cleaning processes that will be required; however, it increases the probability that an impurity may be carried forward into the next step, which could either interfere with that reaction, or even ones that follow later. It might also be more difficult to remove the impurity at a later stage once the desired molecule has different functional groups and properties.

Even if an alternative process can be substituted or used in addition to create smaller particle, crystallization remains the workhorse for making solid forms of APIs that are ready for formulation – and I don't expect this to change any time soon (even if it is considered a "dark art"). What is the key to success? Addressing the crystallization process early in the development cycle offers real benefits for developers in terms of time and costs later during scale-up. Early work can also help in determining an API's chance of success by generating consistent product throughout scale-up, which not only meets purity specifications, but also has the physical properties suitable for formulation into finished drug products. Ensuring a product has the physical properties to make handling and processing on plant is also vital to efficient operations during manufacturing. The upshot? Innovators save time and money.

Unstoppable

Sitting Down With…
Sheila Mikhail, CEO and co-founder, AskBio; founder, Columbus Children's Foundation

How did you come to start your own law firm?

I was practicing law in Boston when my ex husband was recruited by Duke University, so I came to North Carolina with the intention of practicing at one of the largest law firms in the southeast. I was experienced in complex transactions, mergers and acquisitions, and venture capital, but I was told that, as a woman of color, clients would not be comfortable receiving complex legal advice from me. I was asked to stay in the background and advise my peers, who would then be the interface with the client. It was disturbing.

I decided to start my own law firm, Life Sciences Law. Bayer, which was one of my clients at another law firm, transferred with me and underwrote my firm. Over time, the business became very profitable and very successful within the life sciences niche. Our clients included Gilead, Sanofi, GSK, and Bayer. One of the nicest things about starting my own law firm was the flexibility.

How did you get involved with Jude Samulski and gene therapy?

It was fate! When a course was canceled at the University of North Carolina (UNC), somebody had to step in as a guest lecturer just a few hours before it was due to begin. The lecture was to focus on spin outs and licensing from the tech transfer office. I was very knowledgeable in this area so I stepped in to give the presentation. Jude Samulski was in the audience. He approached me after the presentation; he had been trying to get a gene therapy company spun out of UNC, but was finding it difficult. He asked if I would help.

We tried to get a CEO to join the company, but gene therapy was in a kind of a nuclear winter following the death of Jesse Gelsinger. Who would want to work in a company with dismal funding prospects? But I was very interested in the company. Jude was investigating muscular dystrophy and it was obvious that these children had very poor health outcomes. There was no treatment to sustain them so I thought it was a worthwhile mission, so I became a co-founder and the CEO of AskBio.

At the time, Jude said, "I can't pay you." In fact, he didn't pay me for over a decade! I worked at a law firm to put money into the company and Jude took out a second mortgage on his house. Jude said, "If we are successful, we will change the world."

Today, I think we really have changed the world. Jude's technology is now used in Zolgensma and Luxturna.

What were the biggest motivations in the early days?

There were always other options where we could have made bigger financial returns more quickly, but this did not motivate us. Today, I always talk to my employees about working for a purpose. You spend a lot of time at work; if you aren't behind the mission of the organization, you should find something else to do. Drug development is a very risky business, and it's a very hard business. We didn't get our first round of funding until 15 years after we started the company, so you really have to buy into the mission.

Along the way, we came to know a lot about the children that were treated and their parents. They were making so many sacrifices and counting on us. This reality was very motivating and made us realize that we had to keep going.

How did you manage to progress programs into the clinic without funding?

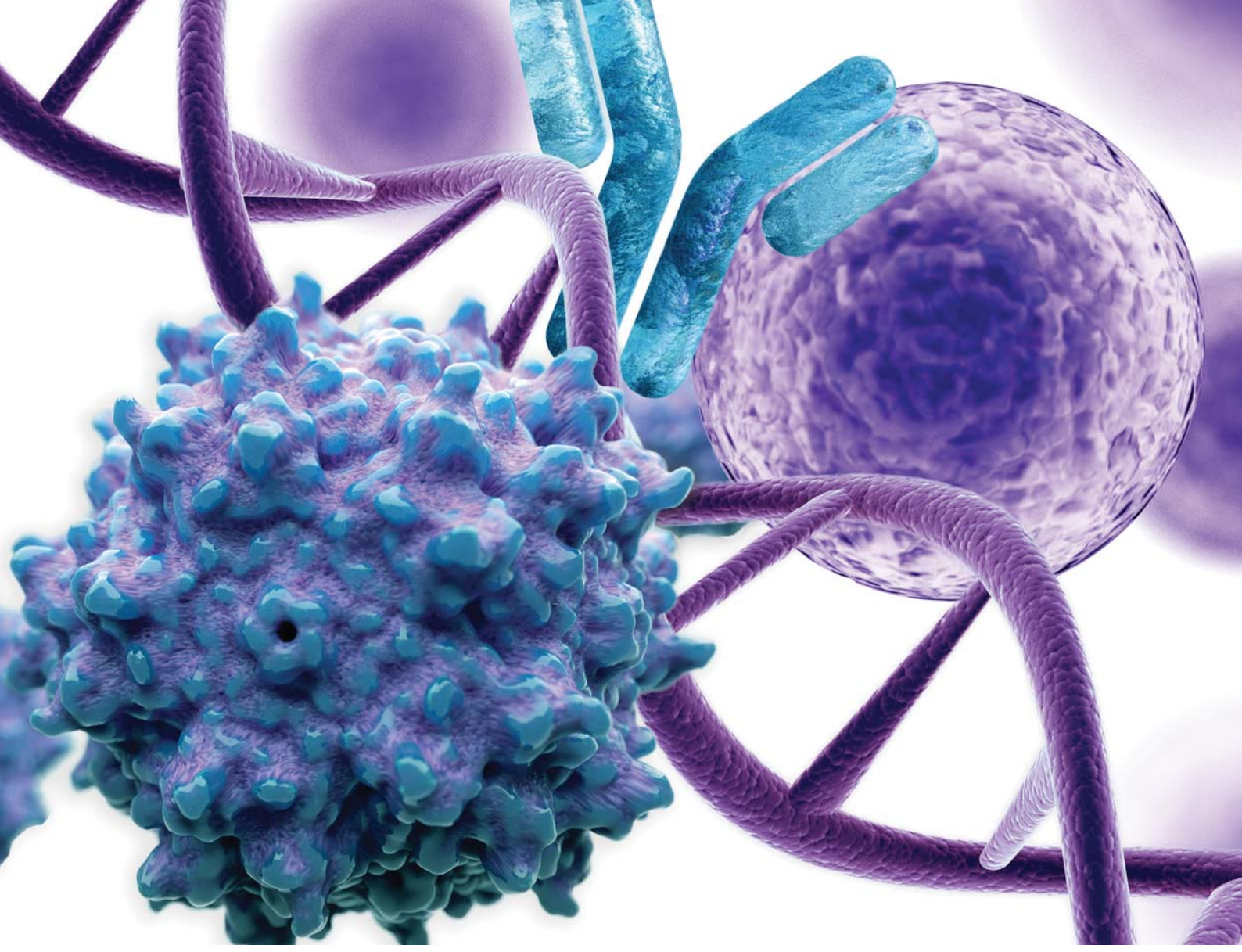
Well, it's a lot easier to do things with money, so we built a CDMO business and added significant manufacturing capacity. I kept saying, "Well, it's only money. If that's the only problem, we'll figure it out." Most companies just stop if they don't have the funding, but we were really insistent on our mission!

"I love the application of science when it changes people's lives."

The Jesse Gelsinger death shut down the gene therapy field and left a cloud over our technology, but we were determined to push through and demonstrate that gene therapies could be safe. We came up with creative strategies, but, when you're bootstrapping a company, it's extremely difficult to get people engaged. We had to find people that were true believers. To this day, I am very grateful to those who stuck with us. There were many times when we almost went into bankruptcy, but we always figured out how to pull it off.

What continues to drive you?

I love the application of science when it changes people's lives. I'm not trained as a scientist, but I read all the papers and I attend all the conferences. It's such an exciting time for molecular medicines. First we had the gene addition augmentation approach, then it became the gene editing approach, then base editing, and now we have prime editing and cancer vaccines. Every year there seems to be a new tool that provides more precise treatment or expands the number of diseases that can be treated. Each of these different tools is right for different diseases. Hopefully, we'll be able to shift from treating disease to preventing disease – using tools such as next generation sequencing to predict what's going to happen as we age – or even when a child is born. It's an incredibly exciting time.



proven expertise & integrated solutions across biologic modalities

Partner with a trusted CDMO and accelerate your biologic to market. From virtual startups to top pharma, we have helped 50+ protein, cell and gene therapy, and sterile injectable products reach the market with our integrated and standalone offerings, local to global reach, and collaborative approach.

- CELL LINE DEVELOPMENT
- PROCESS & FORMULATION DEVELOPMENT
- DRUG SUBSTANCE MANUFACTURING
- DRUG PRODUCT FILL/FINISH
- DEVICE ASSEMBLY & PACKAGING
- STANDALONE & INTEGRATED ANALYTICAL SERVICES

Catalent®
BIOLOGICS

DEVELOPMENT DELIVERY SUPPLY

Comprehensive solutions. Advanced technologies. Better biologics.™

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