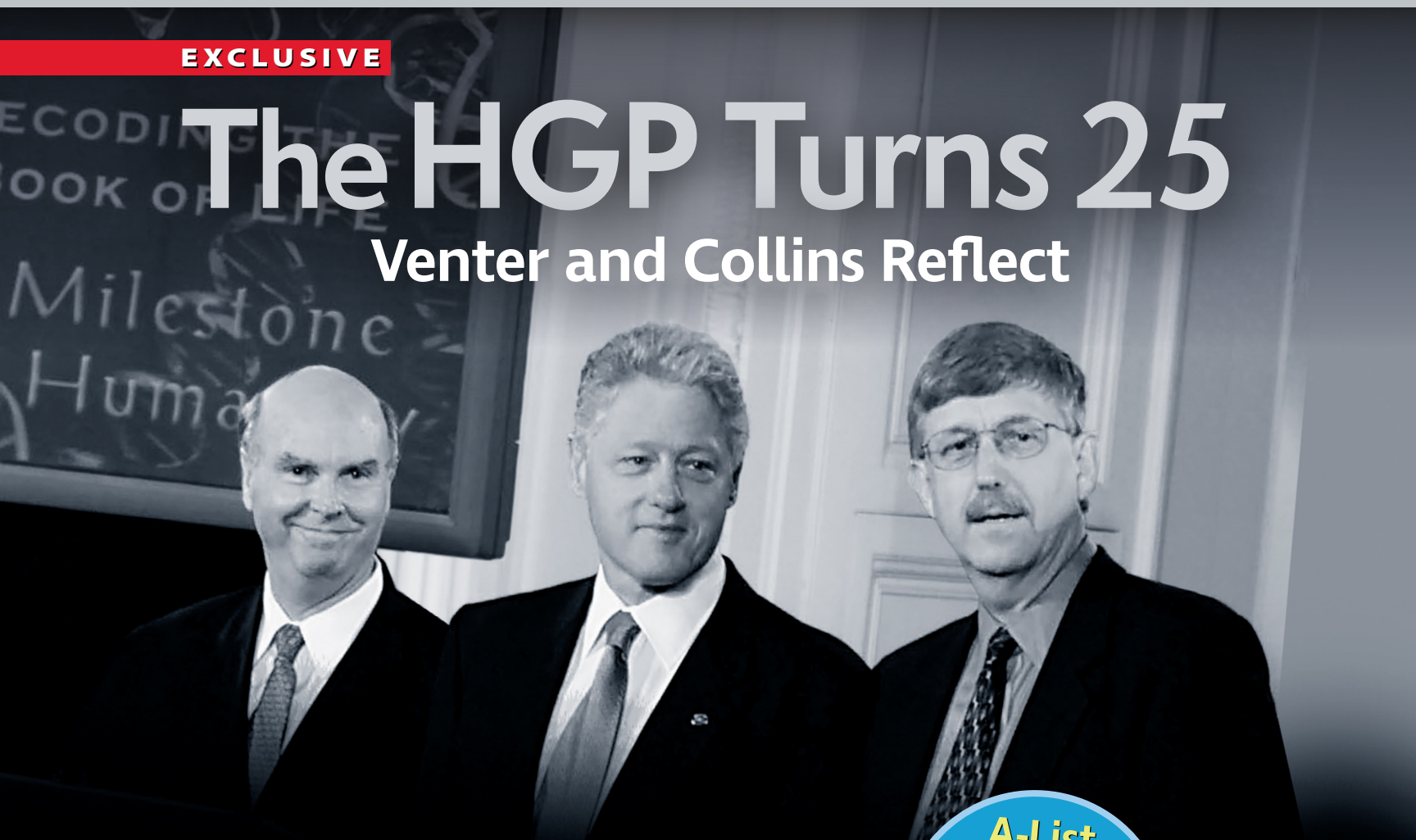
GEN Genetic Engineering & Biotechnology News

JUNE 2025



EXCLUSIVE

The HGP Turns 25

Venter and Collins Reflect

New Animal Alternatives
Evolve on Multiple Fronts

Turning Back the Clock
Regenerating Aging Cells

Proteomic Partnerships
Put Multiomics Into Practice

A-List Top 10 Genome Editing Therapy Companies

Mary Ann Liebert, Inc. publishers

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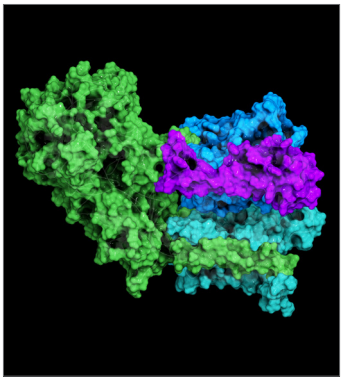

What's **Trending Online** GENengnews.com

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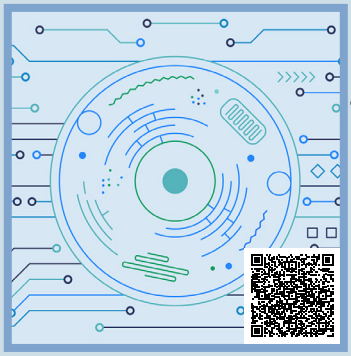
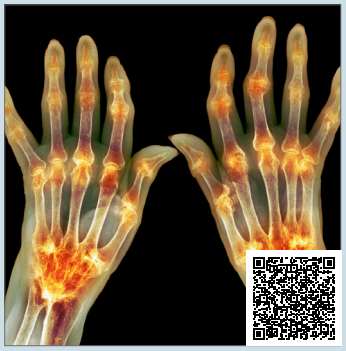
SARS-CoV-2 Likely Spread Through Wildlife Trade, Not Bat Migration



Chan Zuckerberg Initiative

Chan Zuckerberg Initiative Releases TranscriptFormer AI Model



Science Photo Library/Getty Images

Gut Microbiome Linked to Rheumatoid Arthritis Through Reprogrammed T Helper Cells

Cover Image: Mark Wilson/Newsmakers/Getty Images

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GEN Genetic Engineering & Biotechnology News

Humanized Mice: Next-Gen Animal Models Engineered for Translational Research

A fundamental challenge in preclinical research is accurately predicting human responses using conventional animal models. Traditional approaches often fail to adequately model human disease, creating significant translational obstacles between laboratory findings and clinical outcomes.

The Jackson Laboratory's (JAX*) humanized mice offer a sophisticated solution by faithfully recapitulating human physiology and pathology. These advanced models enable more reliable, accurate, and predictive research across applications in immuno-oncology, infectious disease, and autoimmunity.

Redefining Translation Research with JAX Humanized Mice

JAX humanized mice represent a paradigm shift in preclinical research. Through sophisticated humanization processes, human genes, cells, or tissues are strategically incorporated into mouse physiology, creating models that generate *in vivo* responses more similar and translatable to human biology than conventional models. These advanced approaches provide researchers with unprecedented predictive power across diverse research applications, including immuno-oncology, stem cell biology, and therapeutic efficacy and toxicity testing.

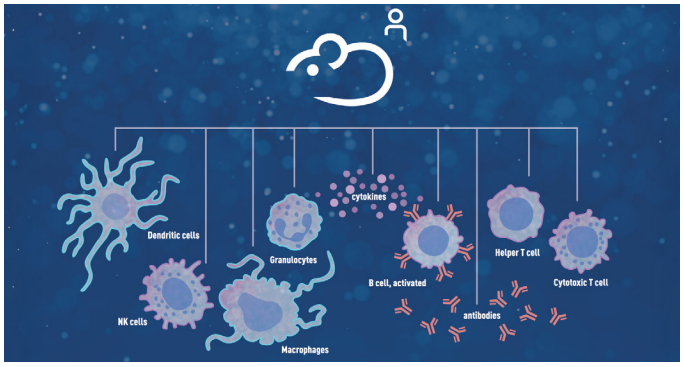
JAX offers a comprehensive portfolio of humanized models, each designed to address specific research challenges:

Immune Humanization

JAX specializes in immune humanized mice, engineered to contain human immune cells, including both naïve hematopoietic stem cells (HSCs) and mature peripheral blood mononuclear cells (PBMCs). These powerful *in vivo* tools provide exceptional platforms for evaluating compounds in immunology, immuno-oncology, infectious disease, and hematopoiesis research.

JAX's NOD *scid* gamma (NSG*) mouse demonstrates superior engraftment of HSCs compared to all other available strains. These stem cells develop into functional human immune cells within the mouse, creating a model that closely recapitulates key aspects of human immunity. JAX's advanced humanized NSG portfolio features strategic modifications to host genes and tissues, delivering representation of specific human cell functions *in vivo*. This platform supports long-term studies investigating complex human immune cell interactions and enables reliable testing of therapeutics with high human specificity.

In PBMC-humanized mice, human immune cells are engrafted into mice lacking a functional murine immune system. Since these engrafted PBMCs matured within a human immune system, they recognize the mouse host as foreign and mount a graft-versus-host disease (GvHD) response to mouse tissues. These models use mature human immune cells from characterized donors known to develop cytokine release syndrome (CRS) when exposed to CRS-inducing therapeutics, making them valuable for both studying GvHD and evaluating the likelihood of compounds triggering CRS in humans.



The Jackson Laboratory

Humanized Mice and the Immune System Components produced and provided by The Jackson Laboratory.

Human Tumor-Bearing and Onco-Hu® Humanized

JAX offers sophisticated humanized models specifically designed for oncology research. Our human tumor-bearing platform features NSG or NRG mice engrafted with human cancer cell lines (CDXs) or human solid/liquid tumors. With a database of over 400 well-characterized solid patient-derived xenografts (PDXs) and 10 acute myeloid leukemia PDXs available for engraftment, these models provide a cost-effective platform for proof-of-concept and target validation studies.

Additionally, our advanced Onco-Hu® models are co-engrafted with both human immune cells and PDX or CDX tumors. This co-engraftment better recapitulates the clinical tumor microenvironment, enabling more efficient investigation of immunomodulatory drug candidates' therapeutic potential.

Atlas™ Mice from AbTherx

In partnership with AbTherx, JAX has developed the Atlas™ mouse—a genetically humanized mouse model that produces chimeric mouse-human antibodies when immunized. These antibodies combine mouse constant regions with human variable regions while preserving the full complement of human diversity. Atlas mice are engineered to generate antibodies with the desired diversity, developability, and affinity required to accelerate the discovery of mono-, bi-, and multi-specific antibodies to help bring life-saving therapeutics into the clinic more quickly.

Through their faithful recapitulation of human physiology and pathology, humanized mice help researchers accelerate their studies and advance drug discovery. ■

*JAX and NSG are registered trademarks in the USA and other countries.

To learn more about JAX's comprehensive suite of humanized models and explore how these models can fit into your research plans, scan the QR code and connect with our scientific team today.

www.jax.org

The Jackson Laboratory

From the **Editor in Chief**

"Only time and money stand between us and knowing the composition of every gene in the human genome." —Francis Crick, 1986

John Sterling

This *GEN* issue celebrates the 25th anniversary of former President Clinton's White House news conference on June 26, 2000, that announced the completion of the first draft of the human genome. Francis Crick, PhD, was indeed prescient about the roles of money and time in what began in 1990 as the Human Genome Project (HGP).

Originally estimated to take 15 years to complete at a cost of $3 billion, the project was finalized two years earlier in 2003 at a cost of roughly $2.7 billion. The results (among others): the HGP facilitated identifying disease-associated genes, understanding disease mechanisms, and detecting genetic predispositions early. The development of personalized medicine and targeted therapies has led to tailored treatments, insights into how individuals respond to different drugs, and cell and gene therapy.

This issue includes *exclusive commentary* from the two principals driving the HGP—Francis Collins, MD, PhD, who ran the publicly-funded government program and J. Craig Venter, PhD, who led the private-sector commercial effort via Celera Genomics. The competition between the two groups was often testy and sometimes nasty. Clinton ordered Venter and Collins to "fix it," and both appeared with the president at the White House event on June 26.

As noted for the section beginning on p.16, *GEN* interviewed eight officials representing either a biotherapeutic, diagnostic, or vendor company to get their thoughts on how the project transformed the life sciences—two called it the biological equivalent of the moon landing in 1969—and how some of the tools and technologies that were spun out of the Human Genome Project supplied the basis for the founding of their company and its associated business strategy.

All provided thoughtful and informative replies, but remarks from the CEO and co-founder of Twist Bioscience, Emily LeProust, PhD, struck me as especially insightful:

> *"Reflecting on the 25-year anniversary of the Human Genome Project, I remember the early hype—people had huge expectations. But 10 years in, many were disappointed. Everything we had predicted was starting to happen—but not on the timeline people hoped for.*
>
> *People overestimate what they can do in the short term, and underestimate what they can achieve in the long term. This is the perfect example. Twenty-five years later, the Human Genome Project is an absolute, smashing success. It's had a profoundly positive impact on human health. But in the short term, the breakthroughs were slow—because science just takes time."*

John Sterling

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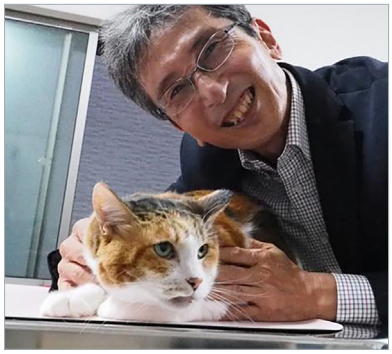
Sticky ends

Garfield's Ginger Genetics Revealed



Hiroyuki Sasaki/Kyushu University

Anyone who studied genetics in college knows something about the calico cat's fur pattern: it is the classic example in any genetics textbook of X-chromosome inactivation. But it has remained a mystery which gene on the X chromosome is responsible for the orange coat color. Now, researchers have found the mutation behind a cat's orange fur. Two papers describing the work are published simultaneously in *Current Biology*. The team analyzed DNA from 18 cats—10 with orange fur and 8 without—and found that all orange cats shared a specific deletion in the non-coding region of *ARHGAP36* gene. Hiroyuki Sasaki, PhD, Professor at Kyushu University's Medical Institute of Bioregulation and the Institute for Advanced Study (and self-proclaimed cat-lover), plans to continue this research, including using cat cell cultures to decipher the molecular function of *ARHGAP36*. Since the gene also exists in humans and is linked to conditions like skin cancer and hair loss, these findings could have surprising medical relevance.

A Flock of Starling Friendships

Friendship comes naturally to people; it's human nature, so to speak. But the idea that other animals make friends has been difficult to prove. Now, a new study suggests that birds demonstrate "reciprocity," or helping each other out with the expectation that the favor will eventually be returned. The new findings, published in *Nature*, draw on 20 years of research conducted on African starlings. The researchers studied thousands of interactions between hundreds of birds and collected DNA from individuals in the population. By combining behavioral and genetic data, they asked the questions: Did the birds preferentially help relatives? Did they help non-relatives even when relatives were available? And did they reciprocate help with specific individuals over the years? The findings show that helpers preferentially aided relatives, but also frequently and consistently helped specific non-relative birds, even when relatives were available to help. "Many of these birds are essentially forming friendships over time," Dustin Rubenstein, PhD, Professor of Conservation Biology at Columbia University said. "Our next step is to explore how these relationships form, how long they last, why some relationships stay robust, while others fall apart."

Dustin Rubenstein / Eureka Alert

fcafotodigital / Getty Images

Chilling a Chili Pepper

Chili peppers are notoriously spicy. Capsaicinoid compounds found in the peppers are to blame. But for some peppers, despite high levels of capsaicinoids, their heat is mysteriously dull. Now, a paper published in ACS' *Journal of Agricultural and Food Chemistry* identified three compounds that lessen peppers' pungency. The researchers collected dry, powdered samples from 10 types of peppers, including Chile de árbol, serrano, African bird's eye, Fatalii, and Scotch bonnet. A trained panel of taste testers evaluated the intensity of the powders in tomato juice. Despite the same amounts of capsaicin and dihydrocapsaicin in each sample, the 10 peppers' perceived heat intensities ranged significantly. The researchers identified compounds (capsianoside I, roseoside and gingerglycolipid A) that dialed back the heat intensity. "These advancements could enable the customization of desirable spicy flavor profiles or lead to the creation of a household ingredient designed to tone down excessive heat in dishes—the anti-spice," says Devin Peterson, PhD, professor in the department of food science and technology at The Ohio State University. "Additionally, they hold significant medical potential in the design of (non-opioid) analgesic agents for pain management."

HOT Off *the* WEB

Biopharma Stocks Rise as Trump Orders Drug Price Cuts

traffic_analyzer / Getty Images

By Alex Philippidis

President Donald Trump signed an executive order to lower U.S. prescription drug prices to their levels in other countries, though many details of the "most favored nation" (MFN) pricing policy remain unclear—and one industry group criticized the approach as "deeply flawed" and hurtful to smaller drug developers.

Trump declared on social media that the cut would deliver savings of "59%, PLUS!," without immediately specifying how the policy would ultimately be carried out once prices are set. "We will bring fairness to America. Drug prices will come down."

Under Trump's order, Health and Human Services (HHS) Secretary Robert F. Kennedy Jr. was directed to set "target" prices for drugs within 30 days, along with Trump's Assistant to the President for Domestic Policy, Vince Haley, and Centers for Medicare & Medicaid Services Administrator Mehmet Oz, MD.

Over 180 days from the date of the order, Kennedy and biopharmas were directed to negotiate prices for drugs individually.

At the end of the 180 days, Kennedy has authority under Trump's order to "propose a rulemaking plan to impose MFN pricing" if no "significant" progress in negotiation has been made including via Section 804(j), which authorizes importing drugs from Canada (and potentially other countries) to lower drug prices for Americans.

"In any case we think implementation will remain contentious and challenging w[ith] litigation, etc.," cautioned Michael J. Yee, Jefferies equity analyst, and five associate colleagues in a research note.

Yee observed that implementation of rules for MFN remained vague but could be carried out via an executive order similar to one that Trump issued during his first term, which applied lower drug prices to Medicare Part B and biologic drugs. That order was blocked by a federal court and was set aside by the administration of Trump's successor and predecessor Joe Biden.

MFN rules could also emerge via Congressional action, Yee added, "This is key as it's unclear to us if negotiations fail after 180 days whether the president and Kennedy have the power to implement MFN pricing without Congressional approval and ultimately legislation."

Reasoning that the MFN policy announced today was better than feared, investors propelled shares of U.S. biopharma giants higher by single digits. Shares of the four largest biopharmas based outside the U.S. (Novo Nordisk, Roche, Novartis, and AstraZeneca) were all but flat, fluctuating less than 2%.

John F. Crowley, President and CEO of the Biotechnology Innovation Organization (BIO), criticized MFN drug pricing as "a deeply flawed proposal that would devastate our nation's small- and mid-size biotech companies—the very companies that are the leading drivers of medical innovation in the United States and the cornerstone of America's biotechnology leadership."

"Importing socialized medicine will not make American's healthier or our economy stronger. It will only serve to empower China and our other adversaries and undermine our economic and national security. Applying other countries' antiquated approach to how they value—and pay—for medicines will stall investment across America's biotech companies, risk access to vital treatments and cures for millions of American patients, and lead to fewer American jobs." GEN

Downstream Bottlenecks are Slowing mAbs Manufacturing

This New TFF Tech Can Help

Validated and reusable holder-less tangential flow filtration device supports multi-use workflows to simplify downstream mAb processing, reduce manufacturing costs

For decades, therapeutic monoclonal antibody (mAb) production relied on traditional batch-based manufacturing using stainless steel systems. But as biopharmaceutical pipelines grow to meet rising demand for these important biologics, downstream processing must evolve to support faster, more scalable production. In response, many companies are rapidly adopting technologies that improve consistency, reduce contamination risks, and accelerate the time to market for new biologics.

This urgency is reshaping how scientists approach tangential flow filtration (TFF), a crucial downstream step in the concentration and purification of mAbs and other biologics including antibody-drug conjugates and viral vectors. While traditional stainless-steel TFF systems are durable and modular, they rely on traditional cassettes and holders that require precise torquing and complex multi-step assembly, which risks inconsistent sealing, operator error, and increases potential product loss or contamination during repeated use or scale up. Overcoming these operational challenges is the focal point for innovations in TFF system design.

Innovation in response to biomanufacturing pressures

Single-use TFF devices are an attractive option for manufacturers seeking solutions that eliminate setup complexity, reduce contamination risk, and streamline workflows. Meanwhile, multi-use TFF devices are evolving to meet mAb's manufacturers' need for efficiency, consistency, and reduced operational burden. While traditional stainless-steel cassette-and-holder assemblies offer durability, they introduce complexity around setup, torquing, and cleaning validation. More recently, the industry has shifted toward the next-generation of multi-use, holder-less TFF designs: self-contained, flat-sheet cassettes that combine the performance of conventional systems with streamlined handling and validated cleaning protocols.

One such example is TangenX® SC, a next-generation TFF cassette device from Repligen for processing mAbs and other biologics. The device's holder-less sealed design, where the membrane, flow channels, and housing are integrated into a single, sealed unit, significantly improves ease of use, performance consistency, and mitigates risk.

Unlike legacy TFF devices, TangenX SC is factory-assembled, pre-sterilized, and validated for uniform pressure distribution resulting in an 80% reduction in process setup as well as reduced decommission time and operator risk. Because TangenX SC's plug-and-play format does not require special hardware or tools, scientists and process engineers can transition quickly between clinical and commercial-scale production. The cassette leverages Repligen's ProStream and HyStream membranes, with the option of five different molecular weight cutoffs. Importantly, TangenX SC was designed with cross-scalability in mind. It uses the same high-performance membranes, similar surface area options, and flow path geometries as legacy cassettes allowing developers to move seamlessly from older designs to SC without reworking core process parameters.

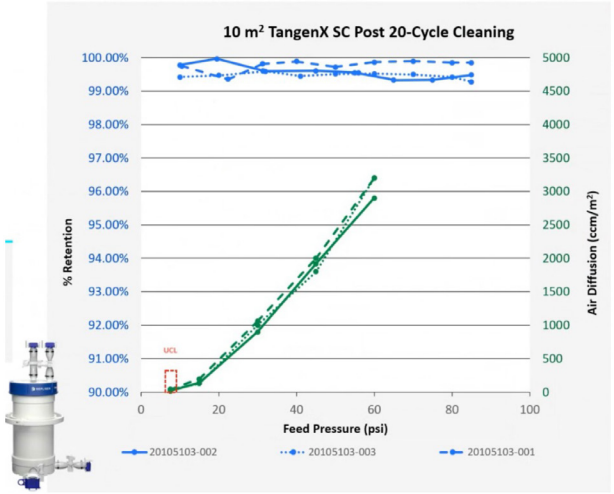


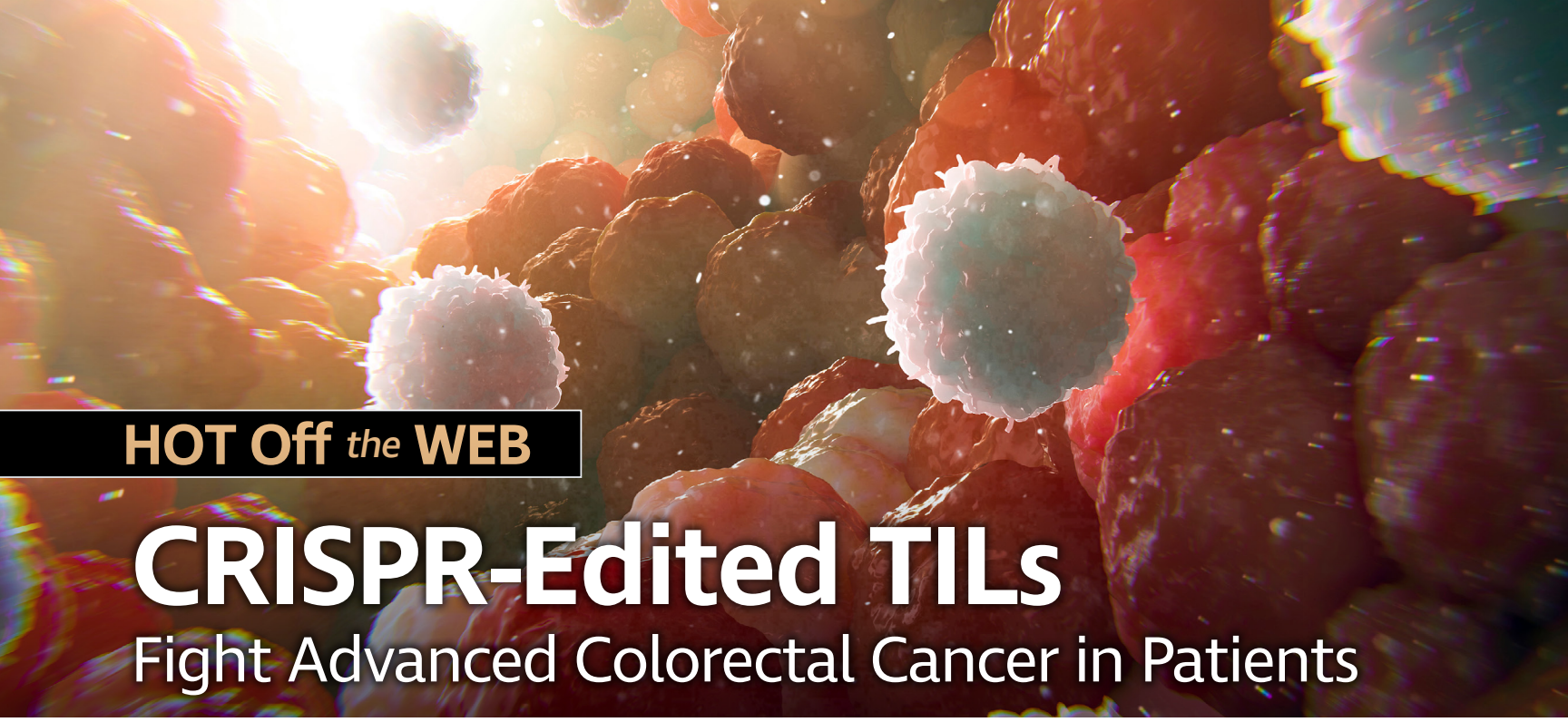
Figure 1.

Repeated use, high recovery across multiple cycles

Performance data on TangenX SC show that it is comparable to traditional cassettes in terms of product recovery, membrane integrity, and process consistency. For multi-use applications, it offers consistent output across batches. Internal tests have shown that the device maintains performance over as many as 20 cleaning cycles under controlled conditions (*see Figure 1*). However, the validated recommendation for multi-use campaigns is up to 10 reuse cycles, based on standard cleaning protocols and routine process conditions. This conservative threshold balances operational efficiency with regulatory expectations for reproducibility and batch-to-batch consistency.

Designed for the future of mAbs manufacturing

The pivot toward closed and continuous systems is accelerating as biopharma companies aim for higher product quality from scalable processes. In an environment where reducing instances of manual handling, open processing, or equipment changeover are essential, adopting TangenX SC can mean the difference between meeting product milestones or missing them. For scientists and process engineers, it means greater control, agility, and productivity in pursuit of therapies that reach patients faster and with higher quality assurance. ■



HOT Off *the* WEB

CRISPR-Edited TILs

Fight Advanced Colorectal Cancer in Patients

Thom Leach /Science Photo Library / Getty Images

Immunotherapeutic strategies have made huge strides in cancer treatment for many solid tumors over the past decade. But the promising results have failed to make a large change in the treatment of gastrointestinal forms of cancer.

Now, a first-in-human clinical trial is testing a CRISPR/Cas9 gene-editing technique to help the immune system fight advanced gastrointestinal cancers. The results show encouraging signs of safety and potential effectiveness of the treatment.

This work is published in *The Lancet Oncology* in the paper, "Targeting the intracellular immune checkpoint CISH with CRISPR-Cas9-edited T cells in patients with metastatic colorectal cancer: a first-in-human, single-center, Phase I trial."

"Despite many advances in understanding the genomic drivers and other factors causing cancer, with few exceptions, stage IV colorectal cancer remains a largely incurable disease," said Emil Lou, MD, PhD, a gastrointestinal oncologist with the University of Minnesota Medical School, Masonic Cancer Center, and M Health Fairview, and clinical principal investigator for the trial. "This trial brings a new approach from our research labs into the clinic and shows potential for improving outcomes in patients with late-stage disease."

In the study, researchers used CRISPR/Cas9 gene editing to modify tumor-infiltrating lymphocytes (TILs). By deactivating the CISH gene, modified TILs were better able to recognize and attack cancer cells. The aim of this study, the authors write, is to "determine the safety and anti-tumor activity of knockout of CISH, which encodes cytokine-inducible SH2-containing protein, a novel intracellular immune checkpoint target and a founding member of the SOCS family of E3-ligases, using tumor infiltrating lymphocytes (TILs) genetically edited with CRISPR-Cas9 in patients with metastatic gastrointestinal epithelial cancers."

The treatment was tested in 12 highly metastatic, end-stage patients and found to be generally safe, with no serious side effects from the gene editing. Several patients in the trial saw the growth of their cancer halt, and one patient had a complete response, meaning that in this patient, the metastatic tumors disappeared over the course of several months and have not returned in over two years.

"We believe that CISH is a key factor preventing T cells from recognizing and eliminating tumors," said Branden Moriarity, PhD, associate professor at the University of Minnesota Medical School, Masonic Cancer Center researcher, and co-director of the Center for Genome Engineering. "Because it acts inside the cell, it couldn't be blocked using traditional methods, so we turned to CRISPR-based genetic engineering."

Unlike other cancer therapies that require ongoing doses, this gene edit is permanent and built into the T cells from the start.

"With our gene-editing approach, the checkpoint inhibition is accomplished in one step and is permanently hardwired into the T cells," said Beau Webber, PhD, associate professor at the University of Minnesota Medical School and Masonic Cancer Center researcher.

The research team delivered more than 10 billion engineered TILs without adverse side effects, demonstrating the feasibility of genetically engineering TILs without sacrificing the ability to grow them to large numbers in the lab in a clinically compliant environment, which has never been done before.

While the results are promising, the process remains costly and complex. Efforts are underway to streamline production and better understand why the therapy worked so effectively in the patient with a complete response in order to improve the approach in future trials.

This study is registered with ClinicalTrials.gov, NCT04426669, and is complete. This research was funded by Intima Bioscience. GEN

InVivoKines, A New Generation of Cytokines for *In Vivo* Research

Optimized long-lasting and high activity Fc-cytokines

The choice of the best protein reagents for *in vivo* research is crucial to ensure good reproducibility and results. Optimized proteins are often developed at pharma or biotech companies for their own purposes. They are not accessible to the research community.

To this end, AdipoGen Life Sciences recently launched a new generation of recombinant fusion Fc-cytokines, called InVivoKines™. They are produced under GMP-like conditions for immunological, immunotherapeutic, and preclinical *in vivo* research.

Cytokines are small proteins that facilitate communication among immune cells and orchestrate the response to infections and tumors as well as overall immune homeostasis, making them attractive for preclinical and clinical research for a variety of immune-related disorders.

However, for *in vivo* experiments, use of recombinant cytokines is limited by their short half-lives in serum, meaning higher doses need to reach the expected effect followed by dose-induced toxicities. To overcome this drawback and use lower amounts of cytokines, half-life needs to be extended while preserving the cytokines native engagement of their respective receptors.

One widely used strategy to increase the cytokine half-lives *in vivo* is to fuse the biologically active protein to the constant fragment (Fc) domain of a human immunoglobulin (Ig) to allow vascular retention mechanisms mediated by neonatal Fc receptor (FcRn) recycling and, therefore, increasing the presence of the Fc-protein in the serum. A typical Fc fusion protein prolongs half-life to 1 to 3 weeks instead of a few minutes/hours for the untagged cytokine.

Many Fc-fusion cytokines are used in research or have been approved as biotherapeutics with a half-life of almost a week(s). However, Fc-fusion frequently leads to daisy-chaining and aggregation during production, severely impacting activity. In addition, using a classical Fc approach leads to dimeric cytokines, while most cytokines are highly active only as monomers.

To solve these issues, AdipoGen utilizes the Knobs-into-Holes Fc technology (KIH), already used to create bi-specific antibodies, to design new long-lasting and highly active Fc-cytokines, called InVivoKines. This Fc-KIH technology allows Fc heterodimerization to create a structure with two different arms to design naturally occurring active monomeric or heterodimeric proteins with conformational stability and FcRn binding.

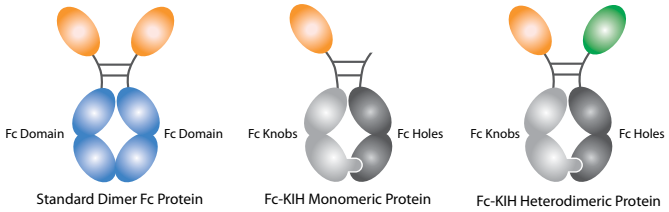


Figure. Structure of classical Fc versus Fc-KIH fused proteins.

Advantages of InVivoKines

InVivoKines show identical activity compared to the endogenous or untagged recombinant cytokines but exert much more activity compared to classical Fc-cytokines that form dimer or higher structures. InVivoKines demonstrated excellent performance when validation testing first began. For example, comparison of mouse IL-21 InVivoKine with the standard IL-21 (mouse):Fc in a T-cell activation assay showed that the IL-21 InVivoKine is the appropriate reagent to study T cells with 500x higher activity compared to the standard IL-21 (mouse):Fc. The monomeric structure of the IL-21 InVivoKine, as well as that of other tested InVivoKines, allows the preservation of the high endogenous activity of the cytokine together with the extended half-life. So, highly active InVivoKines, reagents will help researchers to successfully perform crucial *in vivo* experiments.

Abrogation of the Fc activity by LALA-PG mutations

To avoid any Fc activity during *in vivo* experiments, AdipoGen engineers silenced Fc-KIH domains of InVivoKines using the hIgG1-P329G LALA mutations (called LALA-PG), which completely abolishes FcγR and C1q interactions with unaffected FcRn interactions and Fc long-lasting effect. Thus, InVivoKines with the LALA-PG mutations do not elicit Fc activities such as functional cell-based assays and inflammatory cytokine responses.

In summary, AdipoGen Life Sciences released most cytokines as InVivoKines to provide researchers with the best tools that they will need for *in vivo* experiments in mouse or rat. In addition, since the activity of InVivoKines is identical to recombinant untagged cytokines, these reagents can also be used for *in vitro* assays. ■

Forensic Genomic Analysis: A Force Multiplier for Law Enforcement

By Gail Dutton

Genomic analysis techniques are still relatively new in forensic labs, but when applied they can solve cold cases rapidly

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Focus
A pioneer in applying genomic forensics to crime scene evidence to identify missing and unidentified persons, helping law enforcement agencies resolve cases that would otherwise remain mysteries.

A human skull, hidden between walls, was perhaps the last thing owners of a Batavia, Illinois, home expected to find during a 1978 renovation. Police investigated but, finding nothing, sent the unidentified remains to a university anthropology department. Scientists there determined the person was a woman in her mid-twenties and probably had died before 1900.

In 2021, the Batavia Police reopened the case and in 2023 they brought in Othram, a forensic DNA specialist laboratory, to sequence the DNA and identify potential relatives.

Eventually, the woman was identified as 17-year-old Esther Ann Granger, born in 1848. She had died 157 years earlier, in 1866, and was buried in Indiana before—investigators think—someone exhumed and sold her remains, possibly for medical education.

The Granger case is remarkable because of the age of the DNA they were able to analyze, but the true value of forensic genomic analysis lies in identifying more recent cases involving missing persons and violent criminals. Othram's website tells the stories of more than 500 men, women, and children who disappeared, and whose bodies were identified only because of forensic DNA. The technology is so powerful that the Bureau of Indian Affairs recently announced a joint project with Othram to help identify the remains of missing and murdered Native Americans and Alaskan natives.

"Forensic DNA analysis offers a way to solve cases that are truly unsolvable," David Mittelman, PhD, CEO of Othram, says. "Using genomic tools in forensics is a force multiplier for investigations."

SNPs and degraded DNA

"Forensics inputs are radically different than what you expect to see in the research or clinical setting," Mittelman says. Unlike most clinical DNA sequencing settings, forensic DNA specialists are faced with samples that have been degraded by the elements or preservatives, making the fragments quite short. That, in turn, makes standard analyses using short tandem repeats (STRs) difficult, if not impossible. Instead, Othram's analyses focus on single nucleotide polymorphisms (SNPs), which can be identified using much smaller fragments.

For context, a consumer DNA test collects between about 750 and 1,000 nanograms of DNA material. Othram accurately identified the perpetrator in a sexual assault case from a sample of only 0.12 nanograms.

Below 100 picograms (0.1 nanograms), allele dropout is likely, which means analysts may build a profile without the entirety of the genetic data needed to identify someone, rendering that profile less valuable.

Although Othram can successfully analyze small quantities and seriously degraded DNA, sometimes it must decline law enforcement requests. That's usually because its lab hasn't had success with that type or quality of sample. Rather than risk a limited sample, Mittelman says he would rather reject the sample until a more successful technique becomes available. "We err on the side of caution," he stresses.

The company keeps a database of DNA types and quantities that it has rejected and is working—along with other DNA analysis companies—to develop techniques or technologies that, eventually, will allow it to take on those samples. "When we've validated a new method (in the research lab), then we can bring it to the forensics side," Mittelman says, and reach out to the agencies that have experienced previously rejected samples.

Limited cross-fertilization

"Five years ago, there wasn't much going on in this space," Mittelman recalls. Even today, "There is relatively little cross-pollination between the genomics and forensics communities."

Othram is identifying previously unidentified remains at a rate of more than one every two days, working with law enforcement to solve both ongoing and cold cases.

DNA analysis compares DNA profiles to known reference samples. Genomics analysis is broader, he explains. It uses whole genome sequencing to analyze the remains' complete genetic makeup and may point investigators to research genealogy to help identify victims. Therefore, even when genomic tools exist, forensics teams may not have access to them, or the tools may still need to be optimized for forensics applications.

For Othram, that meant creating a purpose-built forensics genomics lab that "marries a forensics lab with a genomics lab powered by the **Illumina** NovaSeq platform." The company also developed three new technologies: Forensics-Grade Genome Sequencing® (FGGS®), KinSNP® for rapid kinship inferences in near or distant relatives, and the DNASolves® Genetic Database. Adding genetic data supplied by individuals—often who have missing family members—makes definitive identification possible. Now the company identifies missing persons or criminal perpetrators nearly every day.

Today, Mittelman says, "We spend a lot of time working on DNA mixtures," and other imperfect forensic inputs, and trying to find ways to not only make the identification process faster, but also more cost-effective, so more law enforcement agencies—"from the FBI to a local agency that doesn't have a scientist"—can use it.

That said, "The important thing in forensics isn't genotyping," he continues. "It's the ability to detect distant relatives, which allows you to piece together someone's identity. The cross-pollination of these fields is a huge opportunity," Mittelman says.

Forensic Genomics Pioneers

When Mittelman and his wife, Kristen Mittelman, PhD, chief development officer, founded Othram in 2018, DNA analysis and next-generation sequencing already had proven their value, but forensic genomic analysis was quite new. With the extra information it supplied, "law enforcement officers were excited but wondered whether this was science fiction. It's science fact," Mittelman told them, "but getting them to try it initially was a challenge."

Now, with thousands of solved cases, it's obvious forensic genomics works. His message today is that "it doesn't just work for some cases. It can work for all cases."

The issue today is funding. It's important for law enforcement leaders to budget for forensic DNA analysis and for them to allow this technology to be used earlier in investigations, rather than reserving it as a last resort (especially when sample quantities are limited).

Othram works hand-in-hand with law enforcement, not only performing the biological work to build and analyze a profile and identify potential relatives, but also helping crowd-fund the work. "If you can find these pieces of data quickly, law enforcement can find out who is responsible for the crime more quickly," he says, "and minimize the chance they'll prosecute the wrong person." GEN

A Silent Mass Disaster... by the Numbers

Approximately 600,000 people are reported missing in the U.S. each year, and some 4,400 unidentified bodies are recovered annually. Roughly 1,000 of those remain unidentified after one year.

Missing persons, unidentified persons, and unclaimed persons account for approximately 60,000 unsolved cases within the U.S. These cases are cataloged in the National Missing and Unidentified Persons System (NamUs), a national U.S. clearinghouse run by the National Institute of Justice (NIJ). Globally, *The Lancet* reports that millions of bodies remain unidentified worldwide.

Additionally, there currently are more than 300,000 unsolved violent crimes, including sexual assaults, homicides, and robberies, according to the U.S. Council of State Governments' (CSG) Justice Center. In 2022 alone, 63 percent of the reported violent crimes were unsolved, including 77 percent of rapes.

"The NIJ has called this 'a silent mass disaster,'" David Mittelman, PhD, CEO of Othram, says.

Top 10 Publicly Owned Editing Therapy Companies

Aleksandr Semenov/ iStock / Getty Images Plus

While commercial uptake by patients still lags and a recent court decision rekindles a legal battle, clinical successes continue

By Alex Philippidis

Three factors will shape development of gene- and other edited therapies over the coming year and beyond.

First, commercially speaking, edited therapies still see lower than expected uptake among patients, primarily the result of payers balking at reimbursement costs for the treatments, which carry sky-high list prices—though therapy developers say their access programs have kept costs far lower for many of those in need of treatments who are unable to pay. Companies without marketed therapies have struggled to contain costs.

Those realities help explain why several public and private companies ranked by *GEN* reduced their headcounts last year—and why the sixth ranked privately held edited therapy developer, **Tome Biosciences**, went out of business last November, just 11 months after emerging from stealth mode with an eye-popping $213 million in venture capital.

The second influential factor is the long-running legal wrangle over who invented CRISPR gene-editing technology in eukaryotic cells. That battle was rekindled by a May 12 decision from the U.S. Court of Appeals for the Federal Circuit in Washington, D.C., which ordered the U.S. Patent and Trademark Office's Patent Trial and Appeal Board (PTAB) to reconsider its 2022 interference decision, which sided with The Broad Institute of MIT and Harvard in a second challenge to its CRISPR-Cas9 patents from the University of California (UC), the University of Vienna, and CRISPR pioneer and Nobel co-laureate Emmanuelle Charpentier, PhD.

PTAB's judgment and decision in the second interference determined that the Broad Institute, MIT, and President and Fellows of Harvard College had priority over the Regents of the UC, University of Vienna, and Charpentier, who is director and scientific member at the Max Planck Institute of Infection Biology, Berlin—known collectively as CVC—in the invention of a single RNA CRISPR-Cas9 system that functions in eukaryotic cells.

"We vacate the Board's determination as to conception and remand for further proceedings," the PTAB decided. "On remand, we instruct the Board to reconsider the issue of conception in a manner consistent with this opinion."

In response, the Broad Institute issued a statement saying in part: "Broad is confident the PTAB will reach the same conclusion and will again confirm Broad's patents, because the underlying facts have not changed."

However, from a clinical standpoint, therapy developers are showing a growing number of successes. The world's first CRISPR-edited therapy, Casgevy® (exagamglogene autotemcel or "exa-cel"), is slowly gaining momentum based on number of patients being treated and number of treatment centers up and running.

Casgevy's initial developer, **CRISPR**

Therapeutics, is among companies appearing on this A-List, which includes 10 publicly-traded developers of therapies that involve editing of genes, genomes, RNA, and other genetic materials. The companies have been ranked on a composite scale based on:

- **Portfolio**: Number of approved treatments and clinical programs. (Portfolio assessments included consideration of how far the clinical programs had advanced).
- **Cash position**: Cash, cash equivalents, and marketable securities, as disclosed by the companies in regulatory filings and press releases.
- **Market capitalization**: Product of the share price and the number of outstanding shares.

Because most publicly owned edited therapy developers do not yet have products that have reached the market, revenue figures are not included among the criteria used for determining company rankings, though our A-List reports their most recent quarterly revenue figures (mostly first quarter or "Q1").

Just missing the list at No. 11 was **Precision BioSciences**, a developer of *in vivo* gene editing therapies based on its ARCUS® genome editing platform. At the European Association for the Study of the Liver (EASL) Congress held May 7-10 in Amsterdam, Precision presented initial safety data from the Phase I ELIMINATE-B trial (NCT06680232) evaluating its PBGENE-HBV program for the treatment of chronic hepatitis B.

That data showed PBGENE-HBV to be well tolerated with no dose-limiting toxicities and no serious adverse events after multiple dose administrations: "These data support the continued evaluation of multiple dose administrations per dose cohort and higher dose level cohorts of PBGENE-HBV, with the goal of achieving HBV cure," Precision researchers concluded. GEN

1. CRISPR Therapeutics (NASDAQ: CRSP)

Casgevy® (exagamglogene autotemcel or "exacel"), the CRISPR-Cas9 gene edited therapy CRISPR Therapeutics co-developed with Vertex Pharmaceuticals, finished Q1 with $14.2 million, which Vertex collects. Vertex leads global development, manufacturing, and commercialization of Casgevy, indicated for sickle cell disease and transfusion-dependent β-thalassemia. More than 65 authorized treatment centers were activated globally for Casgevy as of May 1, with 90+ patients having cells collected. In May, the company reported positive Phase I data for CTX310™ showing peak reduction of up to 82% in triglycerides and up to 81% in low-density lipoprotein. With $865,000 in grant revenue in Q1, CRISPR Therapeutics led across clinical activity (two additional Casgevy programs and seven programs across five non-Casgevy candidates), cash position ($1.855 billion), and market cap ($3.075 billion).

2. Intellia Therapeutics (NASDAQ: NTLA)

Intellia Therapeutics' MAGNITUDE trial (NCT06128629) assessing nexiguran ziclumeran (nex-z, formerly NTLA-2001), a Regeneron Pharmaceuticals-partnered candidate for transthyretin amyloidosis (ATTR) with cardiomyopathy, is expected to exceed 550 patients by year-end, the company said on May 8. MAGNITUDE-2 (NCT06672237), which assesses nex-z in hereditary ATTR amyloidosis with polyneuropathy, has dosed its first patient, with enrollment set to be completed in 2026. NTLA-2002, a wholly-owned hereditary angioedema candidate, is being evaluated in the HAELO trial (NCT06634420), for which Intellia expects enrollment completion in Q3. With $16.627 million in Q1 collaboration revenue, Intellia places second in clinical activity (2 candidates in 3 Phase III trials) and cash position ($707.1 million in Q1) but fourth in market cap with $816.234 million.

3. Beam Therapeutics (NASDAQ: BEAM)

Beam Therapeutics made history in March by announcing the first-ever clinical genetic correction of a disease-causing mutation for alpha-1 antitrypsin deficiency (AATD) among positive initial safety and efficacy data from a global Phase I/II trial (NCT06389877) of BEAM-302, which has since gained U.S. Investigational New Drug (IND) clearance. Beam recently advanced its second of three clinical programs by dosing the first patient in its U.S.-based Phase I/II trial (NCT06735755) studying BEAM-301 as a potential treatment for glycogen storage disease type Ia. With $7.47 million in Q1 license and collaboration revenue, Beam ranked second in market cap ($1.734 billion) and cash position ($1.22 billion, up 43% from Q4 2024), and fourth in clinical activity (3 candidates, all in Phase I/II).

4. Wave Life Sciences (NASDAQ: WVE)

Wave Life Sciences expects to announce data in Q3 from the 200 mg multidose and single dose cohorts of the Phase Ib/IIa RestorAATion-2 trial (NCT06405633) assessing GSK-licensed WVE-006 in AATD with the PiZZ genotype. The 400 mg single dose cohort is set to report data this fall. In 2026, Wave plans to file a New Drug Application for accelerated approval with monthly dosing of WVE-N531, an exon skipping oligonucleotide for boys with Duchenne muscular dystrophy amenable to exon 53 skipping, following positive data in the Phase II FORWARD-53 trial (NCT04906460). $9.175 million in Q1. Wave ranked third in clinical activity (five trials for four candidates) and market cap ($978.751 million), and fifth in cash position with $243.075 million.

5. Verve Therapeutics (NASDAQ: VERV)

Verve Therapeutics announced positive initial data April 14 from the Phase Ib Heart-2 trial (NCT06164730) assessing VERVE-102 in heterozygous familial hypercholesterolemia (HeFH) and/or premature coronary artery disease. Among 14 participants across three dose levels, a single VERVE-102 infusion yielded dose-dependent decreases in blood PCSK9 protein levels and low-density lipoprotein cholesterol (LDL-C), with a mean reduction in blood LDL-C of 53% and a maximum of 69% seen among four participants in the 0.6 mg/kg dose cohort. Reporting Q1 collaboration revenue of $32.976 million, Verve placed fourth in cash position ($497.077 million), fifth in market cap ($384.207 million), and sixth in pipeline activity with VERVE-102 and a second LDL-C fighting clinical program, VERVE-201 (update expected in 2H).

6. Editas Medicine (NASDAQ: EDIT)

Editas Medicine is doubling down on CRISPR-based *in vivo* gene editing therapies, based on positive preclinical data it shared at the American Society of Gene and Cell Therapy (ASGCT)'s annual meeting. Editas presented *in vivo* data showing targeted lipid nanoparticles successfully delivering HBG1/2 promoter editing cargo to hematopoietic stem and progenitor cells—plus *in vivo*

See A-List on page 53

25th ANNIVERSARY

Silver Anniversary of the Human Genome Project

25 years ago, on June 26, 2000, President Bill Clinton hosted a White House celebration to mark the first draft of the Human Genome Project (HGP). The announcement capped two years of fierce rivalry between the international human genome sequencing consortium, led by Francis Collins, MD, PhD, (National Institutes of Health) and the private takeover spearheaded by J. Craig Venter, PhD, (Celera Genomics). To mark the silver anniversary of this historic moment, *GEN* invited Collins and Venter to offer their reflections on 25 years of genome science (*pages 24 and 28, respectively*). And we also asked eight industry leaders in the genomics space to share their perspectives on this anniversary.

Above. **J. Craig Venter, PhD, *left*, President Bill Clinton, and Francis S. Collins, MD, PhD, The White House, June 26, 2000.** Mark Wilson/Newsmakers/Getty Images

MADHURI HEGDE, PHD — Revvity

Madhuri Hegde, PhD
Senior Vice President
Chief Scientific Officer
Revvity

The announcement of the first draft of the human genome 25 years ago was a pivotal moment that transformed the life sciences. It provided the foundational blueprint for understanding human biology and disease and revolutionizing medical research and the field of diagnostics. After the completion of the HGP, it gave researchers the ability to sequence individual genomes to support the development of tailored therapeutics for an individual's genetic mutation and should in the future continue to provide the basis for targeted therapies and precision medicine.

Revvity, which refocused in 2023 to specialize in diagnostics and life sciences, has significantly benefited from the HGP. By leveraging genome sequencing technologies and other advanced tools, Revvity has driven innovation in disease research, drug development, and functional genomics. The company operates a global network of clinical laboratories (in the U.S., U.K., China, India, and Sweden), conducting extensive genomic sequencing, including in healthy and sick newborns through ultra-rapid sequencing. Revvity, a global leader in newborn screening, can support laboratories looking to expand into newborn sequencing with its NeoNGS portfolio.

Additionally, Revvity's Dharmacon portfolio, plays a key role in functional genomics by developing siRNA reagent libraries for targeted gene silencing. Revvity supports drug discovery by providing tools that help identify drug targets and predict drug efficacy. All these efforts are part of a broader precision medicine approach, integrating diagnostics, therapeutic development, and genomics into a cohesive ecosystem. ■

GILAD ALMOGY, PHD — Ultima Genomics

Since the announcement of the HGP in June 2000, genomic information has become foundational to the life sciences. Today, virtually no area, whether drug development, population health, newborn screening, or oncology, functions without it. And yet, it still feels like we're in the early days of the genomic revolution. Sequencing a single human genome is interesting, but its standalone value is often limited. While some monogenic diseases can be traced to a single mutation, most genomic variation has far more complex, context-dependent effects. The real power of genomics emerges at scale, when we can compare across large populations and link genomic data to electronic health records across broad, diverse datasets.

By the time I founded Ultima Genomics at the end of 2016, it was already clear that the genome was more intricate than initially understood. The protein-encoding exome represents just 1–2% of the genome, yet the remaining 98% is now known to contain vital regulatory, structural, and functional elements. Perhaps most exciting is the growing realization that a genome is not static. As cells divide, differentiate, and age, they acquire epigenetic modifications and somatic mutations. Understanding this dynamic nature is still in its early innings, but it plays a significant role in areas like cancer diagnostics. Liquid biopsy approaches that analyze circulating DNA will become increasingly central to routine medical diagnostics.

At Ultima, we remain committed to dramatically reducing the cost of sequencing so that the benefits of genomics can be accessed broadly, across populations, across geographies, and across the full complexity of human biology. ■

Gilad Almogy, PhD
Founder and CEO
Ultima Genomics

EMILY LEPROUST, PHD — Twist Bioscience

Emily LeProust, PhD
CEO and Co-founder
Twist Bioscience

If you look back at the June 2000 HGP announcement, the impact has been massive—especially in disease detection. Think about early cancer detection, therapy selection, or identifying minimal residual disease after treatment, to catch any recurrence as early as possible. All of that on the detection side wouldn't have been possible without the HGP.

And on the therapy side, whether we're talking about antibodies—VHH, IgGs, bispecifics, ADCs—or gene and cell therapies, or now mRNA, the impact has been equally huge. Just in cancer alone, and more broadly across disease, the contributions have been enormous.

At the same time, reflecting on the 25-year anniversary of the HGP, I remember the early hype—people had huge expectations. But 10 years in, many were disappointed. Everything we had predicted was starting to happen—but not on the timeline people hoped for.

To me, this illustrates something I think about a lot: people overestimate what they can do in the short term, and underestimate what they can achieve in the long term. This is the perfect example. Twenty-five years later, the HGP is an absolute, smashing success. It's had a profoundly positive impact on human health. But in the short term, the breakthroughs were slow—because science just takes time.

We were deeply inspired by what happened on the sequencing side. The Human Genome Project was about reading DNA, and it sparked an explosion in companies developing tools for DNA sequencing—think of 454, Ion Torrent, Illumina.

At Twist, we wanted to do the same thing for writing DNA. We were certain that if reading could be industrialized, then writing could too. In sequencing, you take a sample, put it in a machine, and get a digital file. What we do at Twist is the opposite: you give us a digital file, it goes into our system, and we deliver DNA—a physical product. We're writing DNA from scratch.

Without the HGP, I don't know if we would've had the courage to pursue DNA writing. But seeing what was possible with sequencing gave us the belief that the same kind of innovation could happen in synthesis.

We're working to make traditional cloning obsolete. On our website, you can upload a DNA sequence as a file, and we synthesize it from scratch and ship it to you—for use in diagnostics, drug discovery, or basic biological research.

Even though we write DNA, every piece we produce is verified with next-generation sequencing. We couldn't do what we do without the advances from the Human Genome Project. To write and deliver high-quality DNA, we rely on sequencing technologies to confirm accuracy. It's an ecosystem built on itself. ■

REBECCA CRITCHLEY-THORNE, PHD — Castle Biosciences

The completion of the Human Genome Project (HGP) has had a transformative impact on scientific research. It accelerated the discovery of novel genes and identified new pathways in diseases, including cancer. This milestone also spurred the development of advanced sequencing technologies, gene expression analysis, and bioinformatics approaches, enabling the analysis of vast amounts of data. Traditional analysis methods were no longer sufficient for this volume and complexity, creating a need for machine learning techniques to extract meaningful insights.

Beyond identifying mutations, the project enabled us to investigate biological pathways, uncovering the complex biology of cancer and other diseases. This molecular information is now used in the multigene expression profile and spatial omics tests developed at Castle Biosciences. These technologies in turn help drive innovation in diagnostic, prognostic, and predictive tools, all of which have significantly advanced personalized medicine.

Historically, treatment pathway decisions have been based on broad, population-level clinical and pathology features, which often did not reflect the underlying biology of an individual's disease. Tests like those we offer now allow treatment pathway decisions to be guided by a patient's specific molecular profile.

Castle's evolution began by asking: Where can treatment pathway decisions be improved to extend a patient's life or forgo a procedure or therapy that the patient is unlikely to benefit from and thus avoid unnecessary side effects and costs? And how can we harness the outcomes of the HGP to develop tests that guide more personalized, risk-aligned management decisions? Our first test, *DecisionDx-UM*, was launched for patients with uveal melanoma, a rare but deadly eye cancer. This test is now part of the standard-of-care for newly diagnosed uveal melanoma patients. It measures the expression of multiple genes to determine a patient's risk of experiencing metastasis based on the unique biology of their primary tumor. We now offer a portfolio of five clinical tests for patients with skin cancers, Barrett's esophagus and uveal melanoma, four of which use gene expression profiling technology made possible by the HGP. ■

Rebecca Critchley-Thorne, PhD
Vice President, R&D
Castle Biosciences

JOHN LEONARD, MD — Intellia Therapeutics

The completion of the HGP represents a fundamental breakthrough in biological sciences and human health. It is also a map for the buried treasure that all of us have been looking for as we try to understand how genetics underlies the disease process and human health.

Intellia Therapeutics uses CRISPR technology as the basis of our medicines because of its incredible specificity. We're able to pick out a specific sequence anywhere in the human genome and manipulate the genome in that sequence. Having the map of where to deploy that technology is absolutely foundational to our work. Without the human genome sequence serving as a known map, it would be difficult for us to know where to deploy CRISPR, and Intellia Therapeutics, as we know it today, would be unlikely to exist. ■

John Leonard, MD
President and CEO
Intellia Therapeutics

GUDRUN STRENGEL, PHD — AlidaBio

The impact of the HGP has been truly transformative. It has fundamentally changed how research is conducted and how we access genomic information for healthcare. With sequencing available as a routine tool in labs around the world, the research and application landscape has changed profoundly.

One of the most widely recognized areas of impact is in personalized medicine, particularly oncology. Other critical applications include prenatal testing, while adults can now assess their risk for diseases like cancer through BRCA2 testing. Then there are gene, DNA, and RNA therapies. CRISPR gene editing has opened the door to potentially curing diseases at the DNA level. We already have the first FDA-approved CRISPR-based therapy for sickle cell disease. Researchers are now focused on improving delivery mechanisms and ensuring high on-target precision.

RNA therapies are especially close to my heart, as they are related to the focus of AlidaBio. These therapies manipulate gene expression, using RNA interference (RNAi) or antisense oligonucleotides to regulate which genes are expressed. A newer area of science involves editing RNA itself to correct mutations. I would also highlight synthetic biology—the ability to reprogram bacteria to produce biofuels or drugs at scale, replacing inefficient chemical synthesis—and the growing understanding of the microbiome.

Without DNA sequencing, AlidaBio would not exist. We develop next-gen sequencing and other tools to detect RNA modifications, which represent an epigenetic layer of regulation. The idea of RNA sequencing is rooted in the insight that DNA alone does not provide a complete picture. The human body contains more than 250 distinct cell types, each with its own gene expression pattern governed by epigenetic modifications. Think of this as the second phase of the HGP—understanding how genomic instructions are read, regulated, and interpreted by cells. RNA modifications, impacting protein translation, RNA stability, localization, and RNA splicing, provide nuanced layers of control that allow cells to rapidly shift their gene expression programs (and thus their functional state) in response to stimuli.

Our research depends heavily on the reference genome for sequence alignment but the field still needs much deeper exploration of epigenetic regulation mechanisms. Some people question how "actionable" the human genome really is. The answer is: it is *extremely* actionable—once we understand how cells use and regulate genetic information. ■

Gudrun Strengel, PhD
CEO & Co-Founder
AlidaBio

STEVE BARNARD, PHD — Illumina

One of the ways I like to explain the 2000 HGP White House announcement is through an analogy. It was akin to the first moon landing—a huge milestone, marking the peak of the space race of the 1960s. Fast forward to 2003, when the HGP was essentially completed after 13 years of effort. That moment was the spark that ignited the genomic age. If you look at the major scientific achievements of the past 50-100 years, this one stands out. It was a breakthrough that gave us the ability to truly understand genetics at the human level, a pivotal moment in science and medicine.

It is extraordinary to reflect on how far we have come since 2000 in bringing these breakthroughs to scale. Looking back, we had this perfect wave going on at that time, positioning Illumina to lead what would become the NGS revolution. Illumina was founded in 1998; at the same time, David Klenerman, PhD, and Shankar Balasubramanian, PhD, (University of Cambridge) were working on the foundational chemistry behind sequencing-by-synthesis (SBS), and co-founded Solexa, which developed the Genome Analyzer in 2006. Illumina was also advancing array technology, focused on gene expression and SNP genotyping. This work was crucial for the global HapMap Project.

The HGP provided a vital reference map of the genome, which is where Illumina's decision to acquire Solexa became pivotal. We acquired the chemistry and could actually re-sequence the human genome. This distinction is important: while many technologies today are referred to as "next-gen sequencing", the more accurate term is perhaps "next-gen resequencing." One needs the reference genome to effectively resequence.

Acquiring Solexa and SBS chemistry enabled us to make that reference genome actionable. Illumina already had the essential ingredients to build optical instrumentation and flow cells, coupled with expertise in biochemistry, synthetic chemistry, surface chemistry, and informatics. Together with Solexa's innovative SBS chemistry, we created the synergy to build instruments that would enable us to scale sequencing technologies and leverage the reference human genome.

Since then, Illumina has launched more than 10 sequencers, each based on the principle that if we could resequence at higher throughput, faster, better, and cheaper—with the quality, accuracy, and reproducibility needed for science and medicine—then we could expand the applications of genomics. This approach has led to the creation of a market worth more than $50 billion, with applications ranging from drug and vaccine development to population sequencing to cancer research and diagnostics and agricultural improvements. It is hard to fully grasp how much the genomic revolution has transformed so many fields. It is kind of amazing to think about! ■

Steve Barnard, PhD
Chief Technology Officer
Illumina

MATTHEW KELLINGER, PHD — Element Biosciences

Matthew Kellinger, PhD
Vice President of Biochemistry
and Co-founder
Element Biosciences

The Human Genome Project (HGP) truly was the biological equivalent of landing on the moon. It was a game changer: massive in scale, bold in ambition, and incredibly complex to accomplish. Its completion sparked a new wave of innovation, advancing the technologies we rely on, expanding what's possible, and paving the way for breakthroughs like new cancer therapies and precision medicine.

At the same time, it revealed just how many questions remain unanswered—questions that scientists today are passionately exploring, thanks in large part to the foundation laid by the HGP. At Element Biosciences, we owe a great deal to this milestone. We're much more than a sequencing company. The HGP created an urgent need for innovation—to develop the tools and technologies capable of addressing such a monumental challenge. That push for progress inspires our work today.

In hindsight, the HGP was both elegant and awe-inspiring, yet it relied heavily on brute-force methods. That sparked a wave of efforts aimed at making genomic sequencing more accessible, accurate, and efficient. One of Element's guiding principles is to pursue intelligent science—solutions that are thoughtful, effective, and fast. The tools we've developed are a direct result of that legacy. Being able to sequence a genome in a single day for just a few hundred dollars is an extraordinary leap forward, especially when compared to the time and cost constraints of the original project.

Our focus on multimodal analysis also stems from the evolution of thinking that followed the HGP. As the field advanced, it became clear that while the genome offers a crucial foundation for understanding biology, it is only part of the story. That realization has led us and others to adopt a more integrated approach. Today, we're building tools that can simultaneously analyze RNA and proteins, enabling deeper biological insights and driving the development of better therapeutics.

Ultimately, all of this traces back to the HGP and the immense hope and excitement it ignited—a legacy that continues to shape our mission and our vision for the future. ■

Transforming NGS Library Preparation with Automation and Miniaturization

How core facilities can boost productivity, cut costs, and stay competitive

Advances in next-generation sequencing (NGS) have propelled genomics research to new heights. With the introduction of new sequencing platforms such as Element Biosciences' AVITI and Ultima Genomics' UG 100™, alongside the industry-standard Illumina NovaSeq™, the cost-per-sample for sequencing has plummeted. This progress has resulted in core sequencing facilities having more capacity than ever before.

However, as sequencing becomes more affordable, the cost and complexity of library preparation have increased, making it the new bottleneck in high-throughput genomics workflows. To remain competitive and efficient, sequencing core facilities must prioritize automation, miniaturization, and flexibility in their sample preparation workflows.

The growing challenge of library preparation

Before DNA or RNA samples can be sequenced, they must first be converted into sequencing-compatible libraries through a series of liquid handling steps. When performed manually, this workflow demands significant time, labor, and resources. As a result, core facilities face increasing pressure to deliver high-throughput results while maintaining cost efficiency and data accuracy. Contract research organizations (CROs) offer competitive pricing, making it essential for core facilities to maximize efficiency to attract and retain customers.

Automated liquid handling solutions, such as those provided by SPT Labtech's firefly® and mosquito® instruments, are revolutionizing NGS library preparation. These systems not only increase throughput and reproducibility but also enable miniaturization of reactions—allowing labs to optimize reagent usage and reduce plastic waste.

The benefits of automation and miniaturization

One of the major advantages of automation in NGS workflows is precision. Manual pipetting is prone to variability, which can lead to inconsistencies in library quality and sequencing results. Automated liquid handlers improve accuracy, reduce human error, and ensure uniform library preparation across multiple samples.

Yurchanka Siarhei/Shutterstock

Beyond precision, the mosquito liquid handler enables miniaturized library preparation, allowing users to scale down reaction volumes significantly. By reducing the volume of costly reagents required for each reaction, core facilities can extend their budgets while maintaining high-quality sequencing outputs. Additionally, miniaturization helps lower the environmental impact by reducing the use of plastic consumables—an increasingly important consideration in today's research environment.

Case studies: core facilities leading the way

Several leading genomics core facilities have already leveraged our automated liquid handling technology to enhance their NGS workflows.

At the University of Florida, researchers have implemented miniaturized mRNA-seq library construction using mosquito, which has significantly improved their efficiency and reduced reagent costs. This approach has also had implications for their studies on circadian rhythms, demonstrating how high-throughput sequencing can be streamlined without compromising data quality.

Another case study showcases the miniaturization of DNA library preparation for Illumina sequencing, where our mosquito liquid handler helped reduce reaction volumes without sacrificing accuracy, ultimately leading to significant cost savings.

Staying competitive in an evolving industry

As sequencing technology advances and becomes more cost-effective, the role of core facilities is evolving. To meet the growing demand for high-throughput sequencing services, these facilities must adopt flexible and scalable automation solutions. Our firefly and mosquito instruments offer the adaptability needed to work with a wide range of customers, from academic researchers to pharmaceutical companies, ensuring that core facilities can accommodate diverse applications while maintaining efficiency.

By integrating automated liquid handling solutions, sequencing core facilities can eliminate manual bottlenecks, reduce costs, and improve data consistency. Miniaturization further enhances these benefits, making it an essential strategy for modern genomics labs looking to optimize workflows and stay ahead of the competition.

Looking ahead

The future of genomics lies in high-throughput, cost-effective, and precise sequencing workflows. Automation and miniaturization are the keys to unlocking the full potential of NGS. By investing in automation, sequencing labs can ensure they remain at the forefront of innovation—delivering high-quality results faster, more affordably, and with greater sustainability than ever before. ■

Francis Collins Reflects on Human Genome Project's 25th Anniversary

Alex Philippidis, Fay Lin, PhD, and Kevin Davies, PhD

The reference genome was a "bridge into the future," but there is still much work that needs significant support

Francis Collins, MD, PhD
Former Director, NIH

25 years ago, Francis Collins, MD, PhD, led the international consortium that completed the first draft of the Human Genome Project (HGP), an event marked at an historic White House celebration on June 26, 2000. Earlier this year, Collins abruptly retired from the NIH where he'd served for 32 years, including his historic tenure for 12 years as NIH director.

In April, Collins sat down with *GEN* editors Alex Philippidis and Fay Lin, PhD, and editorial director Kevin Davies, PhD, to discuss the 25th anniversary of the HGP and reflect on the alarming pressure on science and scientists being felt at universities and government organizations. (These remarks have been edited for length and clarity).

GEN: ***This June marks 25 years since you walked into the East Room of the White House with J. Craig Venter, PhD, and President Bill Clinton to announce the completion of the first draft of the HGP. What do you recall as you look back on those events?***

Collins: I reflect on it as having been a wonderful moment of being able to tell the world that we actually now have in hand—and it's publicly accessible—roughly 90% of our own DNA instructions. That does feel like crossing a pretty significant bridge into the future. You don't ever have to go back to where we didn't know that before.

And feeling incredible gratitude on my part for the 2,400 scientists in six countries who labored to make this happen. Not worrying about who was going to get the credit, agreeing to high standards about the accuracy of the information, and to give it all away.

Yes, there was this competition with Craig Venter, having announced in May '98 that his company [Celera] was going to tackle this project with, he claimed, a faster and better way to approach the sequencing. The good part of that was it got people's attention! Now, it's a race, a competition! People got more engaged: who has the yacht and who has the motorcycle? Somehow those became topics of interest, too.

It was at times stressful trying to keep the public project on track when there were certainly some pressures, some of it encouraged by Venter, that Congress might need to step back their support for the public project because it's going to get done anyway. It was partly about the technology, but it was really more about the plan for data access. The public project decided back in '96 that all the information was going to be made available every 24 hours.

[Celera] by necessity, because they had stockholders, was not going to be able to do that. Their product would end up being something you had to pay to get access to. That was an anathema to me and the other leaders of the public project.

Ultimately, the general conclusion of everyone is this is a good thing to have. That is information that anybody who's interested can start to work with those three billion base pairs and figure out how to make sense out of them.

GEN: ***How would you sum up the impact of the human reference genome over the past 25 years, both scientifically and economically?***

Collins: What has been the economic return to the United States of the part that the U.S. paid for, which was roughly $3 billion over 13 years to get that first copy of the genome sequence? The last ROI figure I saw was 141:1, probably a couple years ago. So that $3 billion has turned into probably over $1 *trillion* by now by

objective estimates to stimulate economic growth. Not bad.

If we had an opportunity right now that was like the HGP, would it get started? Probably not. It would get done in China.

On the clinical side, the most obvious direct beneficiary of genomic capacity has been cancer. My prostate cancer had its DNA sequenced, and there was an interesting finding there. If I end up with a recurrence, which I hope I will not, that will guide the choice of what to do next. I would say that pretty much has become the standard of care.

The ability to use genomics for infectious disease is just taken for granted. But if we hadn't had all of the developments during COVID, we would not have been able to discover all these variants that changed everything, the need for redesigning the boosters and all that.

Another thing I like to point at, because it's pretty dramatic, is what genomics has done in the newborn nursery, when the doctors are struggling to figure out what's wrong with the available data. Sequence the genome, do it quick, and you get an answer 40% of the time. That's going to have profound impact on the decision about how to manage that newborn's survival.

GEN: ***Do we finally have a truly complete human genome sequence now, 25 years later?***

Collins: We may finally be there with the Telomere-to-Telomere consortium! What Adam Philippy, PhD, and his colleagues did was really quite amazing, taking advantage of long reads that could get you across repetitive parts of the genome, like centromeres and heterochromatin. Now there's an effort to try to build not just one of those, but the pan genome, so that you could see differences across the world, which may help us with [structural variants] in common disease.

The All of Us program, in addition

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to having some 600,000 whole genomes mostly assembled from short reads, are also doing long reads so that you have complete genome sequences of a few thousand individuals. That has turned out to be a goldmine for people trying to understand structural variants.

GEN: ***25 years ago, you offered a host of predictions for the likely medical impact of the Human Genome Project. What fresh predictions do you have for the next 25 years?***

Collins: Some of the things I predicted happened faster than I expected, but a lot of the applications to common disease, because the landscape of genetic variation turned out to be so complicated, have not come into full bore the way I would have liked.

I would have hoped we would see everybody having their genome sequence as part of their medical record. That model still is appealing, but we haven't quite gotten there yet. I think we will in the next 10 years or so. That would also allow the full application of pharmacogenomics, which has such potential to optimize therapeutics. I certainly hope in the next 10 years, a dramatic set of advances of curing Mendelian genetic disorders by *in vivo* gene editing approaches, including conditions that affect the brain. That will be wonderful to happen.

GEN: ***What is your reaction to the recent layoffs and funding cuts at NIH, FDA, and other organizations?***

Collins: The situation is rather dire. The dismissal of many rank and file scientists at NIH, staff and support people, thousands of individuals without a clear indication of both justification or the choices for how those dismissals are happening has left the institution reeling.

A terrible loss has been that of Eric Green, MD, PhD, who with great energy and creativity led the National Human Genome Research Institute (NHGRI) for 15 years and yet was suddenly let go. The institute then ended up being led by his deputy, Vence Bonham, PhD, but only a week later he was also let go. The speed with which these changes are being made and the lack of planning about how exactly the research is still supposed to go forward is deeply troubling.

The head of the Infectious Diseases Institute, Jeannie Marrazzo, MD, was also let go. So it's a time at NIH of a great deal of demoralization. Can the mission still go forward with all of the damage that's been done? We're in a dark period.

Those young scientists in my former [NHGRI] group are the people I was most worried about. They are representative of a whole generation of young scientists who now are in a vulnerable place, many wondering if their dreams of a career in biomedical science are feasible in this country, or whether they have to think about alternatives, perhaps moving to Europe or Australia, to be able to have the support they're going to need to live out those dreams.

GEN: ***Also leaving is Peter Marks, MD, the director of CBER at the FDA, who was held in high regard by the cell and gene therapy community.***

Collins: The loss of Peter Marks was a deep tragedy for anybody who cares about vaccine research but also about cell and gene therapy. I worked closely with him during Operation Warp Speed, when those vaccines were developed in just 11 months. He was absolutely heroic in that circumstance and did many things that people thought FDA wouldn't be able to achieve in the timetable. His leadership of CBER has made it possible for things to happen that might have taken much longer.

GEN: ***A week after you retired from NIH, you spoke at the March for Science in front of the Lincoln Memorial, stating you were "worried for our country." What did you mean by that?***

Collins: I wrote a book called *The Road to Wisdom*, which was motivated by my growing concern after spending 12 years as NIH director watching the state of polarization of our country get worse and worse, even in the face of the worst pandemic in more than a century.

I'm particularly worried because [medical research] is an area I care deeply about, but it goes beyond that. Put science and politics together, you got politics. I'm a person of faith and yet I'm deeply troubled to see many faith communities taking on positions that seem like the opposite of what their faith foundations would have passed on.

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"I am feeling incredible gratitude on my part for the 2,400 scientists in six countries who labored to make this happen." *Francis Collins*

GEN: ***You served as NIH director from 2009-2021. What advice do you have for Jay Bhattacharya, MD, the new NIH director?***

Collins: I'm glad Bhattacharya is there because there were some pretty awful things that happened in the first two months after the inauguration under the oversight of an acting director who didn't really have the ability to do much except follow the guidance that was coming down from HHS and the White House.

Bhattacharya is a Stanford professor and previous NIH grantee, a distinguished MD economist. My hope is that he'll be able to put NIH back in the less political part of the discussion and begin to focus again on what the mission is and how to get there, even in the face of what is right now a major crisis facing the institution. He will have the chance to become the champion for the traditional and visionary goals of NIH, to save lives, reduce suffering, and to use the most visionary kind of rules and technologies to get there.

GEN: ***What are your future plans now that you have left the NIH?***

Collins: I'm still trying to sort that out. I'm trying to do what I can to provide information to the public about why medical research is such a critical part of the future for all of us. and how we need to be sure that it doesn't get damaged in some serious way by what's happening right now. If that is going to reduce our chances of finding a cure for Alzheimer's disease, is that really what we were getting when we decided to vote for this administration?

I understand how to get information in front of people that moves hearts and minds. And it's usually the patient stories that people need to hear more. GEN

Francis Collins was interviewed for "The State of Multiomics & NGS," a GEN *virtual event broadcast on April 23, 2025. The full version of this interview is freely available to view on demand:* bit.ly/StateMultiomics2025.

Epitranscriptomics: A New Frontier in RNA Epigenetics 25 Years After the Human Genome Project

Chemical RNA modifications reveal regulatory mechanisms beyond genetic sequence

This year marks the 25th anniversary of the publication of the Human Genome Project, a monumental scientific achievement that has transformed healthcare and laid the foundation for modern genomics. Since its completion, whole-genome sequencing has become routine and powerful bioinformatics can relate DNA sequence to health and disease. For example, sequencing cancer genomes from tissue and body fluids has ushered in a paradigm shift from treating cancer based on its tissue of origin to identifying the best treatments based on driver mutations and genotype.

Despite this progress, many common diseases such as Alzheimer's, heart attack, and stroke remain poorly explained by DNA variation alone. These conditions lack clear genetic biomarkers, limiting the opportunity to identify risks and to make informed health decisions before serious disease can develop. Moreover, DNA sequence alone does not explain the diversity and adaptability of the more than 200 different cell types of the human body.

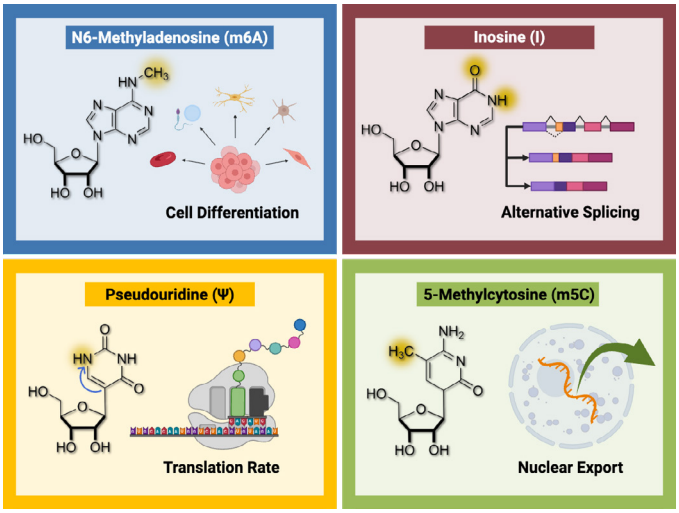
A key missing link is epigenetic regulation, which comprises biological mechanisms for chemically annotating genetic information carriers and controlling when, where, and how the genetic code is expressed. These mechanisms include DNA methylation and histone modifications, both involved in transcription regulation, and RNA modifications, which act dynamically to influence protein translation and other aspects of RNA biology. The entirety of chemical modifications of RNA in a cell is referred to as the epitranscriptome.

Over the past five years, interest in epitranscriptomics has surged due to its role in cellular development and disease, as well as its potential in diagnostics, therapeutics, and biotechnology.

All human RNA species carry subtle chemical modifications, which have large functional consequences. In tRNA, 20% of all bases are modified at conserved positions to support protein-like function. In mRNA, the most abundant and well-studied modifications are N6-methyladenosine (m6A), pseudouridine (Ψ), inosine (I) and 7-methylguanosine in the cap. These modifications are dynamically regulated by three classes of enzymes: writers, erasers, and readers, which install, remove, and bind RNA modifications.

The principal m6A writer, METTL3, is known to be essential for embryonic stem cell development and differentiation, while FTO (fat mass and obesity-associated protein), a known eraser, was linked to obesity before it was implicated in RNA demethylation. Most RNA modifications are silent in conventional RNA sequencing; however, when academic labs introduced the first sequence-resolved detection methods in 2012, it prompted a global effort to uncover their functions and therapeutic potential.

Epitranscriptomics is already transforming biotechnology and medicine. In agriculture, genetic manipulation of m6A pathways has been shown to increase certain crop yields by up to 50%. In medicine, novel therapies are entering clinical trials. STORM therapeutics has developed METTL3 inhibitors for the treatment of myeloid leukemia, with early results showing tumor regression and good tolerance. Wave Therapeutics has launched its first trial for therapeutic adenosine-to-inosine RNA editing, using this RNA modification to correct a mutation causing alpha-1 antitrypsin deficiency.



The four most prevalent naturally occurring chemical modifications in RNA, m6A, Ψ, I, and m5C, influence many key aspects of RNA biology depending, in part, on where they are located in transcripts. One example of a key function is shown for each modification.

RNA modifications are also emerging as biomarkers for multi-omics diagnostics companies. In a landmark for the field, Katalin Karikó and Drew Weissman received the 2023 Nobel Prize for discoveries on RNA base modifications that enabled mRNA vaccine development.

These examples are driven by technologies that read RNA modifications during RNA sequencing. Approaches to RNA modification sequencing include pull-down by antibodies, modification-specific chemical or enzymatic conversion, and direct nanopore sequencing. We are now entering a new era of discovery with commercial platforms such as EpiPlex™ from AlidaBio.

The EpiPlex assay uses barcoding and engineered molecular recognition to encode RNA modifications as part of NGS library preparation. These barcodes are then detected and quantified using the EpiScout™ analysis software. As these tools gain broader adoption and enable discoveries across diverse fields, we anticipate the next 25 years will build on the genome project's legacy, unlocking new insights into human biology through the lens of RNA modifications. ■

J. Craig Venter Describes a Human Genomics Revolution Still In Progress

By J. Craig Venter, PhD

Despite profound impact on biomedical research, progress in understanding has been slow

J. Craig Venter

June 26, 2025, is the 25th anniversary of the White House announcement of the first sequencing of the human genome, and July 28, 2025, marks the 30th anniversary of the publication of the first sequenced genome of a living species, *Haemophilus influenzae*.[1] These anniversaries are more closely linked than might be imagined.

In 1994, Ham Smith and I submitted a grant application to the NIH genome center to use our new idea of whole genome shotgun sequencing to rapidly sequence a bacterial genome. The reviewers and NIH genome leadership were certain that the approach would never work, and the grant was not funded.[2] At the time NIH and DOE were funding a 7-year project to sequence the *E. coli* genome from hundreds of mapped small clones.

The Human Genome Project (HGP) was proceeding on the same distributed clone sequencing approach due to the view that genomes were too complex and had to be broken down into a large number of smaller projects distributed around the world. We surprised and shocked both the genome and broader scientific community with our publication of the *H. influenzae* genome in Science on July 28, 1995, based on a single whole genome shotgun sequencing effort.

I was certain that our approach would work to sequence the human genome, but I had to argue with the editors of *Science* to include the final sentence in the paper, "Finally, this strategy has the potential to facilitate the sequencing of the human genome." [1]

However, at the time, there were only a small handful of scientists, at best, who might have agreed with me. Fortunately, one of them was Mike Hunkapiller, PhD, of Applied Biosystems, now part of **Thermo Fisher Scientific**, who was developing a new capillary DNA sequencer and offered me $300 million to start a company (**Celera Genomics**) to sequence the human genome using my method and his sequencer.

History demonstrates that it was a smart bet; my team sequenced the first human genome in less than one year, and Applied Biosystems made a fortune. I knew that Celera was substantially ahead of the HGP and we had plans to announce our success when President Clinton asked me to consider making the announcement from the White House along with the HGP and declaring that the genome race finished in a tie to end all the public acrimony between Celera and the HGP.

I made the controversial decision to agree to this plan so that the Celera success would not do harm to the public funding of science. On June 26, 2000, almost exactly 5 years after the publication of the first genome, Celera and the HGP announced with President Clinton and British Prime Minister Tony Blair the first versions of the human genome sequence, that were published the following year.[3,4]

The good news

In the quarter-century since Celera and the HGP delivered the first human genome sequences, the world has witnessed a profound

and pervasive genomic revolution. What began as a bold scientific quest—sometimes hyped as the key to "life's blueprint"—has matured into concrete outcomes that touch many aspects of society. The global biotechnology industry has been transformed, growing exponentially and spawning technologies that were unimaginable in 2000.

Drug development has been made smarter and more efficient, yielding therapies informed by our genes and even cures for diseases long deemed incurable. Medicine has become more personalized, with genomics empowering doctors and patients to predict and prevent illness in ways that improve outcomes and quality of life. Innovative companies and business models have risen (and some fallen), all learning how to create value from the code of life.

Meanwhile, governments and international bodies have crafted new policies to support innovation and protect individuals, ensuring that genomic advances proceed ethically and equitably.

Genomics caused what I call a silent revolution that changed both basic sciences and pharmaceutical development. You would have had to be working in science before the 1990's to even remember how slow progress was before you could just search a database for a gene or protein of interest. Projects were usually a decade or longer to isolate a protein and eventually clone the corresponding gene. Nobel Prizes were given out a gene at a time.

Expressed Sequence Tags (EST), rapid gene discovery[5] and genomics changed the decades into a few seconds of computer time. The pharmaceutical industry was almost instantly awash with new potential therapeutic targets and the world changed from searching for a drug target to validating them.

Money for research and investment capital went from a trickle before 2000 to a flood after the White House announcement. The economic impact of genomics has been enormous. In the first 20 years (1990–2010) an estimated $800 billion in economic activity was generated in the U.S. alone. By 2019, human genomics was contributing around $250 billion per year to the U.S. economy and supporting close to one million jobs.

This prosperity wasn't confined to one country—it reflects a global industry transformation, evidenced by the proliferation of large-scale genome initiatives across continents.

Many countries built national biobanks and sequencing programs (e.g., U.K. Biobank's 500,000 genomes; France's "Médecine Génomique 2025"; China's Precision Medicine Initiative). These programs drive local biotech growth and ensure that genomics is truly a global enterprise, not just a U.S. effort.

The genomics sector's value is now measured in the trillions: the global biotechnology market (much of it genomics-driven) was valued around $1.3–1.7 trillion in the mid-2020s. In short, the first human genome sequences triggered a seismic expansion of the biotech industry worldwide—launching new companies, creating jobs, and training a generation of genomics experts.

Genomics became integral to the fabric of biomedical research, medical practice, and society, moving beyond the lab to commercial and clinical sectors.

The not-so-good news

Although several countries launched big-budget programs, the 2016 announcement of China's multimillion dollar investment in its Precision Medicine Initiative is leveraging the country's vast sequencing capacity and population and dwarfing U.S. funding.

Perhaps due in part to budget limitations, the planning at NIH and other agencies was shortsighted in its policies and, as a result, after 25 years the understanding of the human genome has progressed far less than it could have. We still have a limited understanding of how our genetic code has produced over 9 billion unique individuals.

In my view, this slow progress can be attributed to three factors.

1. Short read sequencing technology

Ironically the cost of sequencing genomes and the development of new faster, cheaper technologies while democratizing DNA sequencing has had critical unintended consequences. The first two versions of the human genome published in 2001 were sequenced using Sanger sequencing, which was slow and very costly. Celera's genome cost about $100 million and the HGP around $6 billion. Sequencing large numbers of humans was not going to be feasible at these costs.

Major reductions in genome cost came from new technologies largely driven by **Illumina**. Sequencing costs were driven dramatically down to less than $500/genome by 2024. However, the new sequence technology resulted in short reads of only 1-200 bp, making genome assembly impossible.

As a result, the definition of a genome sequence changed from an independently produced "sequence", to a sufficient number of the short reads that could be layered onto a reference genome to discover SNP variations between the short reads and the reference genome.

Independent assembly of actual genome sequences only restarted recently with the tremendous advances in long reads from single molecule sequencing developed by **PacBio** and an independent approach by **Oxford Nanopore**.

In 2007 the first diploid human genome sequence was completed[6] by the **Venter Institute**. This project, called the *Homo sapiens* Reference Genome Project, produced a high-quality genome sequence that included both sets of phased chromosomes inherited from each parent. The phasing was accomplished by sequencing a number of individual sperm (haploid) cells. For the record this was my genome.

This was a major milestone following the first two human genome efforts, which produced a composite (haploid) reference genome assembled from multiple individuals rather than representing a single, complete diploid genome.

The first diploid genome was significant because it revealed the genetic variations between the two chromosome sets, highlighting the importance of sequencing diploid genomes to fully capture individual genetic diversity. The diploid genome was sequenced using Sanger sequencing and cost an estimated $40 million. Even though substantial genetic variation was not in SNPs but in larger insertions and deletions, the cost led it to be largely ignored other than as the reference to align the new short read sequences.

With short read sequences layered on a reference genome, allele-specific effects were lost by collapsing maternal and paternal alleles, generating a non-existing in nature sequence that obscured and complicated variant interpretation.

For example, compound heterozygotes, where a different gene sequence was inherited from each parent, created an artificial construct showing both variants on a single protein sequence that in reality did not exist. In addition, knowing which parent a trait was inherited from is critical in risk assessments.

2. Missing heritability

This issue finally came to a head after examination of short-read genomes when it was discovered that genetic variants of up to 50% of known heritable traits were missing from SNP data.[7] Heritability estimates from twin or family studies suggest that traits like height, BMI, or schizophrenia, are 40–80% heritable.

Common genome-wide association study SNPs typically explain only 10–50% of total heritability depending on the trait. This should not have been a surprise based on the first diploid genome that showed around one quarter of genome variation was in insertions and deletions of greater than a single nucleotide and that there were more total base pairs in the structural variations than in all the SNPs.

Although the NIH-led telomere-to-telomere (T2T) Consortium proclaimed in April 2003 that the human genome sequence was now completed, the first publication of a complete, phased, T2T diploid human genome actually appeared in July 2023 in *Cell Research* by a team led by researchers from the Chinese Academy of Sciences.[8] This genome assembly represents the first publicly available instance where both parental haplotypes of a human genome were fully resolved from telomere to telomere. The researchers utilized advanced sequencing technologies, including PacBio HiFi and Oxford Nanopore ultra-long reads, combined with Hi-C data, to achieve this comprehensive assembly.

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3. Lack of phenotype data

Many seemed to think that just sequencing large numbers of genomes would make deep understanding and new knowledge fall into place. While that has been true for ancestry tracing and population genetics, without detailed comprehensive phenotype data to accompany genome sequencing little true progress will be made. Much of the genetic data derived from the genome is misleading or just wrong.

For example, APOE4 mutations have been claimed to be diagnostic for predicting Alzheimer's disease. I am a heterozygote for APOE but a brain MRI and Amyloid PET were both completely negative.

As a result, I started **Human Longevity** to do comprehensive imaging and phenotyping along with genome sequencing.

After close to 10,000 individuals screened, not one single APOE heterozygote had any Alzheimer's indications and 20% of homozygotes, including some in their mid 90's also had no Alzheimer's indications. Similar findings occurred with breast and ovarian cancers, where family history is a much stronger predictor than existing genetic markers.

To me this means the causal mutations are part of the missing heritability. We did however find that about 50% of "healthy" individuals had a major tumor or disease that they were unaware of.[9]

Looking ahead

After 25 years, the field of human genomics is now starting over with the right technology to do full diploid phased genomes. So, we are at the point where we can actually have, and test, genome changes in place of just a collection of short snippets if DNA.

The phasing will enable knowing which parent the traits were inherited from, enabling true genealogy of disease and traits. From starting Human Longevity, hundreds of similar centers have opened to help with pre-symptomatic screening, while at the same time creating a set of comprehensive phenotyping datasets to relate back to the genome sequence data.

This must be the future of genome research if we are going to make progress in truly understanding the role our genetic code plays in helping to determine our phenotypes and diseases. GEN

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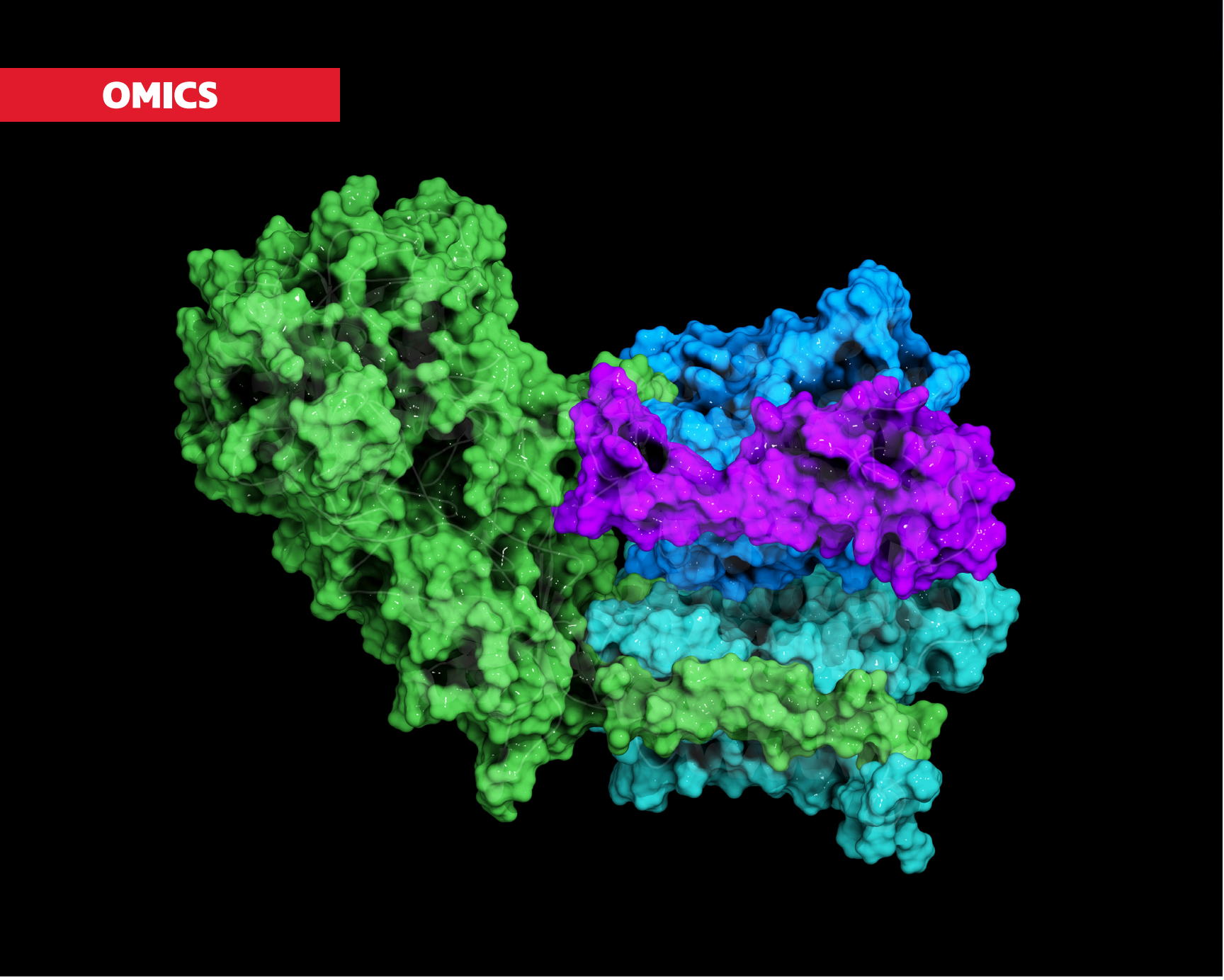

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Proteomics Partnerships Move Multiomics from Theory to Practice

Recent collaborations highlight a growing interest in building a multiomics-based understanding of health and disease

By Uduak Thomas

Multiomics is in vogue and several sequencing companies have taken notice. Rather than building capabilities for things like single-cell sequencing, spatial transcriptomics, and proteomics themselves, some established vendors have opted to acquire existing companies or partner with current providers of these emerging technologies. "What you need to look for has expanded dramatically," David Peoples, **Ultima Genomics** CFO and CBO, told *GEN*. Nowadays, to really understand biology, the scientific community needs to merge DNA with data from the transcriptome, methylome, proteome, and more.

Proteomics in particular has seen a lot of activity, especially in recent efforts to combine gene sequencing and proteomics technologies in a single assay run. Three years ago, Ultima Genomics announced a collaborative effort to pair its sequencer with the Explore HT proteomics platform from **Olink Proteomics**, a **Thermo Fisher Scientific** company. That same year, **Illumina** signed a co-development agreement with **SomaLogic**, now part of **Standard BioTools**, to integrate its SomaScan proteomics assays with Illumina's next-generation sequencing platforms and informatics toolsets. Both proteomics technologies are built so that existing DNA sequencing technologies can also be used to also measure protein identities and expression.

Olink's proximity extension assay (PEA) technology uses an antibody-based immunoassay that features matched antibodies with unique DNA tags and couples these constructs with a polymerase chain reaction to measure thousands of proteins in a single sample. Meanwhile SomaLogic's SomaScan technology uses so-called slow off-rate modified aptamers (SOMAmers), which rely on modified nucleotides to bind to proteins and form complexes that can be captured and quantified using established DNA sequencing technologies.

Fundamentally, sequencing technology can be "an extremely powerful reader of genetic signatures," Peoples said. That capability is turning out to be important for the readouts from these emerging proteomics technologies. And, if the scientific community continues the trend towards multiomics, Peoples believes that sequencers could have a central role in reading the output from other kinds of omics tools beyond the proteome.

A population-scale perspective on proteins

In 2019, neuroscientist and geneticist Chris Whelan, PhD, (currently the director of neuroscience, data science, and digital health at **Johnson & Johnson Innovative Medicine**) was studying the dynamics of protein biomarkers like neurofilament light chain (NfL) and growth-associated protein 43 (GAP-43) in neurodegenerative diseases. Increased levels of these proteins in the blood and cerebrospinal fluid are biomarkers for the progression of disorders like Alzheimer's disease and Parkinson's disease. Whelan's challenge was reliably measuring the levels of biomarkers on a large scale. To do that, he would need a population-sized dataset and tools for measuring thousands of proteins easily and efficiently.

Around this time, Whelan began using new proteomics tools from Olink and SomaLogic in his research and was impressed with the results. The confluence of those two activities led to something of a "lightbulb moment," he tells *GEN*. Could affinity-based technologies be applied to study proteins in the UK Biobank? And would other biopharmaceutical companies be interested in partnering and funding this kind of broad-scale study?

Whelan's instinct was right. In 2020, the U.K. Biobank Pharma Proteomics Project (UKB-PPP), a consortium of some of the biggest biopharma companies in the world, launched with 10 partners. As of January 2025, four more companies have joined the consortium. The current partners are **Alden Scientific, Amgen, AstraZeneca, Bristol Myers Squibb, Calico Life Sciences, Roche, GSK, Isomorphic Labs, Johnson & Johnson, MSD, Novo Nordisk, Pfizer, Regeneron,** and **Takeda**.

As a feasibility study, the UKB-PPP conducted a pilot project with a subset of the UK Biobank data that started in spring 2021 and concluded in winter 2022. The group analyzed nearly 3,000 circulating proteins from 54,000 UK Biobank participants. They have published several papers about their work, including in *Nature* in 2023.[1]

Ray Chen, Olink's senior director for

David Peoples
CFO, CBO
Ultima Genomics

Chris Whelan, PhD
Director of Neuroscience, Johnson & Johnson Innovative Medicine

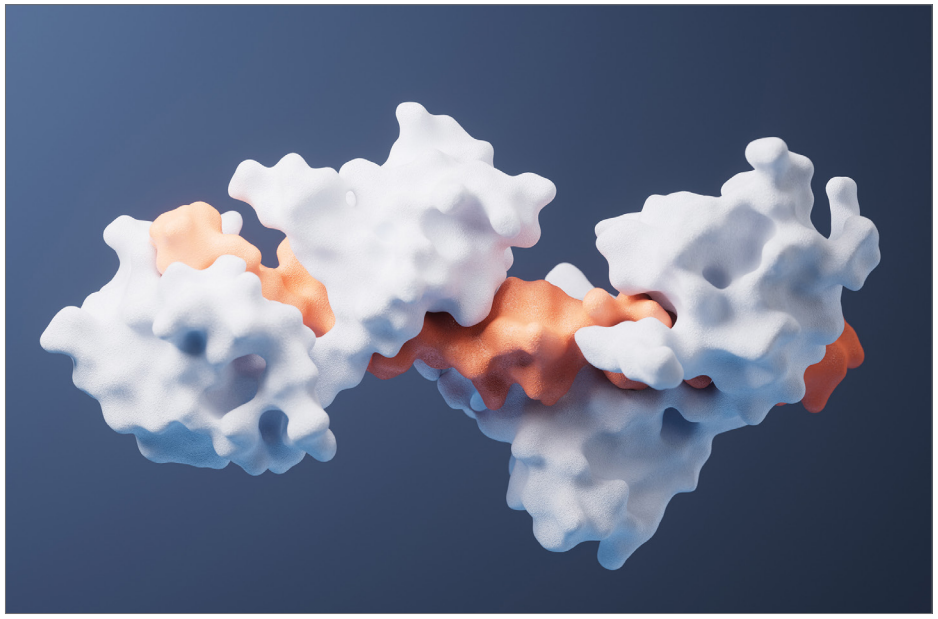
global strategic accounts, presented some of the findings from that pilot study and the *Nature* paper at the 2025 annual meeting of the U.S. Human Proteome Organization (US HUPO). In a conversation with *GEN*, Chen explained how Olink's PEA technology identified more than 14,000 protein quantitative trait loci, linking common genetic variants to changes in protein expression levels. Eighty-one percent of these links were previously unknown, providing fodder for new studies into potential targets for new therapies or diagnostics. The consortium has since published two more papers based on the pilot data and has had their work cited in hundreds of scientific papers.

Dalia Daujotyte, PhD
Senior Product Manager, Illumina

Fiona Kaper, PhD
Vice President, Head, Assay R&D, Illumina

"We've identified probably hundreds of new therapeutic targets [that are] starting points from which we can build new drug programs," Whelan says, including targets for Parkinson's, Alzheimer's, and schizophrenia. "We're applying AI on the proteins directly to find new insights into different kinds of complex diagnoses like major depressive disorders." For example, "We are finding evidence that there may be an immuno-metabolic subtype of depression, but also one that involves dampened mitochondrial activity and one that involves heightened mitochondrial activity."

Buoyed by the success of the pilot, the partners are now moving on to a much larger study of about 600,000 samples, which covers all the people currently in the U.K. Biobank cohort plus some repeat sampling, to examine how protein levels change over time. "It's going to be around 500,000 participants at baseline, but then about 100,000 of those people will have follow-up samples taken somewhere between 7-10 years after that initial visit," Whelan explains. This time, scientists will measure 5,400 protein markers across all samples.



In a pilot study of 54,000 UK Biobank participants, members of the UKB-Pharma Proteomics Project linked over 14,000 genetic variants to expression changes in 3,000 circulating proteins. Jian Fan / iStock / Getty Images Plus

The larger study will use Ultima's sequencing technology paired with Olink's protein detection and measurement platform. After considering biological breadth, throughput, scalability and other insights from their experiences with both platforms in the pilot, about half of the consortium members wanted to proceed with Illumina Protein Prep and the other half wanted to proceed with Olink's offering. Ultimately, the decision to go with Ultima and Olink's technologies came down largely to the complementarity of the Olink PEA technology with the UG 100 sequencer, Whelan says. It also made sense to continue using Olink's assays, as these were also used for the pilot study.

Furthermore, some consortium members felt that working with a different partner could foster a more competitive sequencing market that would only benefit the scientific community. Ultima's Peoples echoes those sentiments regarding healthy competition among tool providers. "I think it's a really good thing for the community that there's competition now out there for these types of projects," he says.

"To be clear, both Illumina and Ultima were very strong partners during the negotiation process and the consortium was happy with the data that Illumina delivered during the pilot phase of the project," Whelan stresses. "As the project leader, it's paramount to me that everything is done in a highly democratic and fair way. It is funny that, on at least two major decisions, we've come to a tie where we need to have a tiebreaker vote. Often it comes down to just very minor factors that help us lean more towards one technology or one path forward, versus the other."

Dalia Daujotyte, PhD, senior director,

product management at Illumina, notes that at the time the UKB-PPP consortium was assessing proteomics partners for the broader project, Illumina's protein assay was still in early testing and could only detect 6,000 proteins rather than the 9,500 it can measure now. They had some internal verification data on the assay but nothing they could show externally at the time. "It was basically a year too soon for Illumina Protein Prep," she says. Also, the consortium had already worked with Olink on the initial 50,000 sample pilot study so it was understandable that they wanted to continue with the technology they began with.

Members of the UKB-PPP consortium were expecting to get the first batch of samples shipped from the UK Biobank in March 2025. They expect to receive the first tranche of proteomic data from 150,000 samples by September 2025, with a second batch of data from another 150,000 samples anticipated in the first half of 2026. The third and final tranche of 300,000 samples will be delivered later. "That's primarily because the consortium has secured funding for those first 300,000 samples," Whelan explained. "We are looking for additional commercial or philanthropic or public partners to help fund the generation of data for the last 300,000 samples."

As the data comes in, members of the consortium will have a limited time to take a first crack at analyzing it for projects they are interested in—they had six months with the pilot data before it was made available to the broader scientific community. They will do joint analysis projects with the data as well, Whelan tells *GEN*, although exactly what those will look like is still being discussed.

Illumina's proteomics play

Illumina has initiated a separate pilot project to also analyze proteins from 50,000 UK Biobank samples using Illumina Protein Prep and the NovaSeq X Plus sequencer. Collaborators with Illumina are **deCODE Genetics**, Standard BioTools, **Tecan**, GSK, Johnson & Johnson, and **Novartis**. "We wanted to set our own standard using Illumina Protein Prep," Daujotyte says. "The U.K. Biobank is a trendsetting biobank. A lot of scientists reuse their data for many different applications so it's a very important biobank to work with."

Like the UKB-PPP, this pilot will analyze 50,000 samples that span different disease types and health statuses. The partners have agreed that deCODE Genetics will process the samples using the Illumina Protein Prep solution and NovaSeq X Plus system and run analysis using Illumina's DRAGEN Protein Quantification pipeline.

> **Nowadays, to really understand biology, the scientific community needs to merge DNA with data from the transcriptome, methylome, proteome, and more.**
>
> —David Peoples,
> CFO, CBO, Ultima Genomics

The first 30,000 samples are funded through co-investment from Illumina, deCODE Genetics, and Standard BioTools, with automation support from Tecan. The data from this initial batch of samples will be available immediately to the scientific research community once the data has passed quality control. This is expected to happen in the second half of 2025. Data from the remaining 20,000 samples will initially be available to the pharma partners for a limited period before being made available more broadly.

For Illumina, moving into proteomics now makes sense given the growing interest in a multi-omics approach to biologics development. "This is part of the broader strategy to lead the next wave biologics discovery," says Daujotyte. Illumina Protein Prep is the sequencing vendor's first proteomics solution. A key differentiator is the fact that Illumina is offering an end-to-end solution for protein discovery from sample collection through sequencing and data analysis.

As of press time, Illumina Protein Prep was in early-access testing with a full launch planned for later this year. The technology uses the SOMAmer technology developed by SomaLogic, which currently measures 9,500 unique proteins in a single assay, the most of any other affinity-based proteomic platform. Illumina is positioning it as a solution for protein discovery that includes sequencing and data analysis capabilities, which Daujotyte says sets it apart from the competition.

Another differentiator is the aptamer technology. There are benefits to using oligonucleotides, says Fiona Kaper, PhD, vice president, head of assay research and development at Illumina. "These can be made and remade over and over again, very reproducibly," she explains. "So, you get that continued reproducibility over different batches."

Furthermore, "you don't have to start splitting up your sample into multiple reactions because you can do it all in one go," which contributes to its reproducibility, she continues. That's important because "the more you split up samples, the more variability you insert in the assay." GEN

Reference

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New Approaches to Ischemia-Reperfusion Studies

Alternatives to *in vivo* model systems using atmospheric control

Introduction

Alternative model systems to study ischemia-reperfusion are needed to support a wide range of disease-related investigations in the life sciences. Ischemia and reperfusion are relevant to many disease states ranging from the impact of diabetes on the retina to stroke, cardiovascular disease and kidney dysfunction.[1,2] However, the current approaches for these types of study are resource-intensive and require greater throughput. Here we examine how alternative *in vitro* methods that combine atmospheric control systems with the throughput offered by microplate readers can provide a boost to ischemia-reperfusion research.

Ischemia-reperfusion injury

Ischemia-reperfusion injuries occur when the blood supply returns to a part of the body after it has been cut off for some time. The initial lack of circulation or ischemia can damage the cells and tissues of different organs due to deprivation of oxygen and nutrients. In addition, the restoration of blood flow or reperfusion can cause further damage due to oxidative stress and inflammation. The events that take place during ischemia reperfusion contribute to the pathological changes linked to atherosclerosis, stroke, high blood pressure, and heart attacks. For the most appropriate clinical intervention, it is important to understand the physiology behind ischemia and reperfusion injury. In addition, studies are needed to identify and develop new drugs for unmet medical needs.

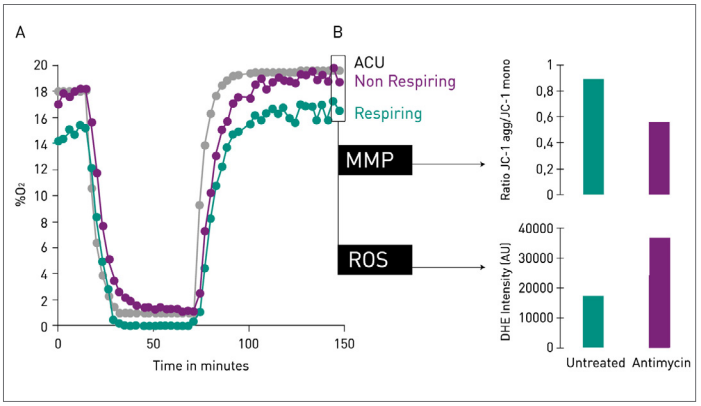

Figure 1. *In vitro* ischemia-reperfusion experiment with multiparametric analysis of respiring and non-respiring iPSC-derived cardiomyocytes. (A) Cell oxygenation monitored with MitoXpress Intra through a hypoxia and reperfusion cycle. (B) MMP and ROS generation analyzed with JC-1 and DHE, respectively, for respiring and non-respiring cardiomyocytes.

Microplate readers: fidelity for ischemia-reperfusion studies

What qualities do microplate readers equipped with atmospheric control systems offer for ischemia-reperfusion studies? At the top of the list is their ability to control the gas environment quickly and effectively under conditions of high fidelity with *in vivo* model systems. Many microplate readers can control CO_2 and O_2 levels in the range of 1–20%. In some cases, they can decrease O_2 to 0.1%. The CLARIOstar® *Plus* with Atmospheric Control Unit (ACU) is the only microplate reader on the market that can rapidly reoxygenate the measurement chamber of the reader to simulate reperfusion by active venting. In addition, the CLARIOstar *Plus* can reach 0.1% O_2 and can read at 100 datapoints per second. Here we highlight a few examples where this level of performance can enable physiological studies and drug development.

Mimicking the *in vivo* environment through atmospheric control

Researchers need to study the behavior of tissue-specific cells under conditions that closely mimic the *in vivo* environment. One way to study the intracellular oxygenation level of these cells in real time is to use the MitoXpress Intra probe (Agilent Technologies).

In *Figure 1A*, oxygenation levels of iPSC-derived cardiomyocytes were monitored through a hypoxia and reperfusion cycle. The CLARIOstar *Plus* with ACU facilitated precise atmospheric control from environmental O_2 levels encountered in the laboratory to 1% oxygen in less than 10 minutes (*grey curve*). This was followed by a defined time at 1% oxygen followed by a rapid reperfusion (gas ramping) to 18% O_2 (*Figure 1A*). Cells were treated with antimycin to block mitochondrial electron transport and inhibit cell respiration. The non-respiring cells reflect the conditions produced by the ACU. Untreated respiring cells (*green curve*) reflect the significantly reduced O_2 concentrations. The extent of respiration by the cells markedly impacts the oxygenation concentrations at the cell monolayer. Cells that are actively respiring experience lower resting oxygen concentrations and a more sustained period of hypoxia.

Another distinct advantage of a microplate reader system is the ability to perform different assays in parallel (multiplexing). In this example, multiplexing on the CLARIOstar *Plus* permitted analysis of the mitochondrial membrane potential (MMP) through determination of the JC-1 aggregate/monomer ratio (JC-1 is a fluorescent membrane-permeant dye that exhibits a shift in emission color from green

to red as the MMP becomes more polarized). In addition, generation of reactive oxygen species (ROS, *Figure 1B*) were detected using the dye dihydroethidium (DHE). Oxidative stress could be readily measured in parallel to the oxygenation levels within the cells.

Drug-related applications

Scalable, precise, and cost-effective ways like those offered by microplate readers and ACUs are not only needed to study cellular behavior but also to look for possible drug interventions.[3]

Hypoxia chambers have typically been used for ischemia reperfusion studies in the past but are limited in throughput and lack endpoint assays for measurement. These limitations have driven interest in model systems that rely heavily on the use of experimental animals. As demonstrated in the previous example, microplate readers enable kinetic assays to be performed in parallel under closely defined conditions of ischemia and reperfusion. Researchers can probe oxygen-induced stress, apoptotic cell death and other metabolic changes in detail. Significantly, due to the speed and fidelity of the gas ramping capabilities of the ACU, kinetic monitoring is enabled, which creates possibilities to look at how different drug interventions impact the consequences of ischemia reperfusion events in real time. These drug-related applications can be studied in cell experiments on a microplate reader. Small molecule drugs can be screened for protective effects against ischemia reperfusion injury including the study of specific outcomes on cell signaling events and processes like apoptotic cell death.

Ferroptosis is a type of cell death that is characterized by iron-dependent oxidative damage to membrane phospholipids. The extent of lipid peroxidation due to ferroptosis is impacted by ischemia reperfusion. Researchers therefore need ways to mimic the *in vivo* environment to study possible interventions for the diseases that arise from ferroptosis including neurological conditions.

In the study described here, lipid peroxide levels were quantified under conditions of normoxia and ischemia reperfusion at 37°C. Lipid peroxides were readily quantified using fluorescence measurements on a microplate reader in the presence of the lipophilic fluorescent probe Bodipy 581/591 C-11 (*Figure 2*).

Oxygen concentrations ranged from 1-18%. A significant increase in lipid peroxidation levels was observed after 2 hours of ischemia and 24 h of reperfusion in SK-N-DZ neuroblastoma cells. The administration of RSL3, a selective ferroptosis inducer and/or the ferroptosis inhibitor ferrostatin-1 during reperfusion reduced production of lipid peroxides. The CLARIOstar ^{Plus} with ACU readily enabled oxygen levels to be manipulated to replicate the conditions of ischemia reperfusion in these experiments using neuroblastoma cells.

In vitro assays that accurately mimic the *in vivo* environment were therefore a useful alternative to classic *in vivo* approaches for measuring increases in lipid peroxidation after ischemia reperfusion and studying the implications for disease.

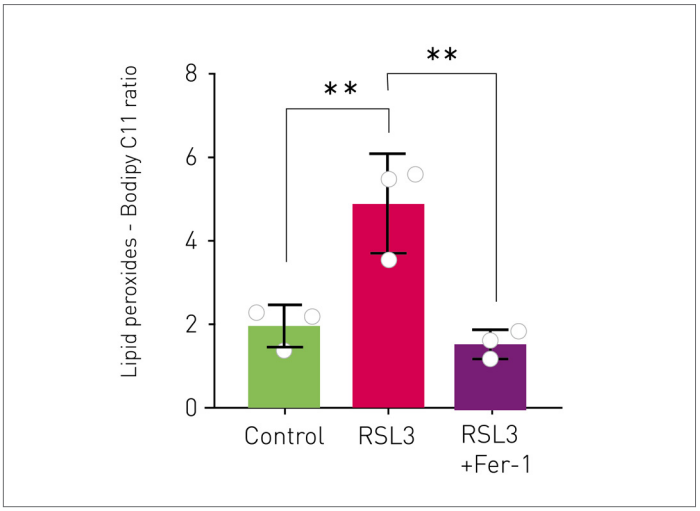

Figure 2. Detection of lipid peroxidation using Bodipy 581/591 C-11. Mean with SEM (n=3). Each experiment has 6 replicates. One-way analysis of variance (ANOVA) with Dunnet's test for multiple comparisons. ** P value < 0.0076. RSL3 is a selective ferroptosis inducer; Fer-1 is ferrostatin-1, a potent and selective ferroptosis inhibitor.

Conclusion

The shift away from animal model systems depends on the availability of physiologically relevant cell models that can also translate into a drug discovery platform. Gas ramping capabilities that go hand-in-hand with the speed and accuracy offered by microplate readers offer distinct advantages to researchers, including a substantial increase in throughput. The CLARIOstar ^{Plus} with ACU allows relevant cell models to be cultured under *in vivo* conditions by mimicking not only hypoxic conditions but also reperfusion. The fast sampling rates and multiplex capabilities support the generation of reliable data for studies of the underlying mechanisms of disease and the development of new interventions. ■

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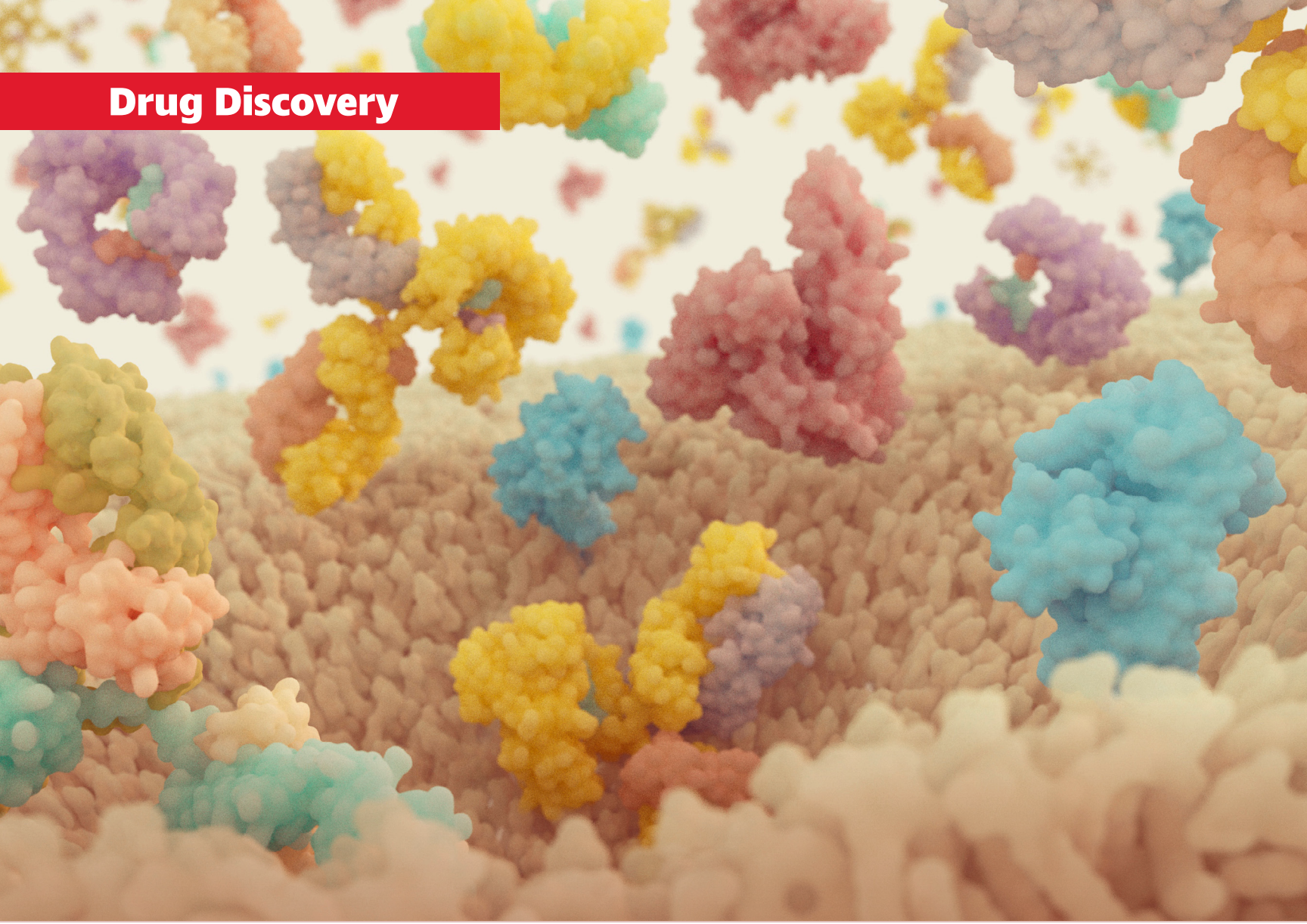


New Translational Models

for Drug Development Forge Ahead

Both small animal models and microphysiological systems gain in sophistication and value as they expedite *in vivo* preclinical testing

By MaryAnn Labant

Small animal models are still a mainstay of preclinical research. The most significant current trend in model generation focuses on immune system humanization of these models to improve efficiency and translational benefits, along with expanded offerings for neurodegenerative diseases.

As genetic-engineering tools mature, vendors are providing additional flexibility for select applications with custom model generation and preclinical services for in-depth characterization.

Another major evolution in the preclinical tools landscape is the growing acknowledgement and acceptance of NAM (non-animal models). The use of NAM is often a first-line testing strategy to confirm or uncover new findings before moving on to the relatively more expensive small animal models. This approach also facilitates adherence to the 3Rs (Replacement, Reduction, and Refinement).

As the NAM industry matures, vendors offering a range of preclinical *in vivo* tools are learning how to work hand-in-hand to provide exponential value to drug developers. This next-generation biology will likely shape drug development in the next few years, highlighted by the April announcement by the FDA to phase out animal testing requirements for monoclonal antibodies and other drugs.

Pushing the boundaries

According to Sierra Kent, PhD, associate director at **The Jackson Laboratory** (**JAX**), JAX pushes the edges of possibilities for humanized mice. A prime example is the NSG-FLT3-IL15 mouse, the world's most advanced model for cellular humanization with CD34+ human hematopoietic stem cells (HSCs).

This strain carries a knock-out of the mouse Flt3 receptor with transgenes expressing human FLT3 ligand and human IL15. When engrafted with HSCs, the NSG-FLT3-IL15 mouse produces a cellular-diverse human immune system, including development of myeloid cells, mature natural killer (NK) cells, functional dendritic cells, and T cells. JAX provides this strain pre-humanized with HSCs along with preclinical services.

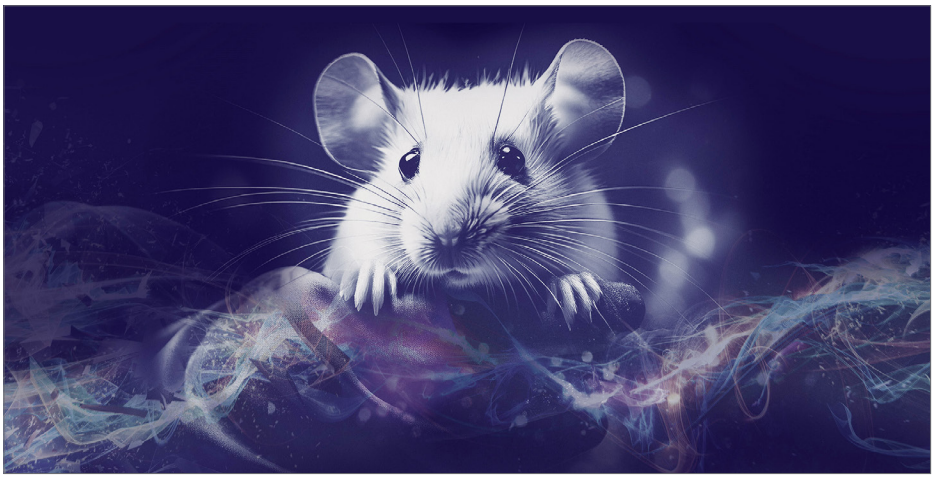
The genetically humanized FcRn mice provide translational pharmacokinetics (PK) and pharmacodynamics (PD) data for antibody-based therapeutics. The Tg32 hALB mouse, lacking the mouse Fc gamma receptor (Fcgrt) and albumin (Alb) while expressing the human counterparts, expands this collection. Tg32 hALB mice are the first of their kind for studying the PK and PD of human albumin therapeutics as well as human IgG and Fc-domain based therapeutics. Preclinical services using these models are available.

This year, JAX expects to make available mouse models with functional human B cells, models for studying autoimmunity, and a variety of pre-characterized human PBMC humanized models.

The over 500-line human iPSC repository also continues to grow at JAX, with engineered iPSC lines carrying disease-relevant mutations associated with neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, ALS, and frontotemporal dementia. In partnership with the iPSC Neurodegenerative Disease Initiative (iNDI), JAX is engineering additional iPSC lines to enable reproducibility studies by introducing the same variants across genetically distinct backgrounds.

"We believe that advanced cellular models, such as iPSC-based platforms, serve as powerful complements to traditional *in vivo* models," said Kent. "While some findings are consistent across systems, others uncover unique biology, underscoring that no single model fits all research questions."

New supply chains and mouse propagation locations fuel a global expansion. JAX Mice® are currently propagated outside of the U.S., in Japan at JAX Japan, in India via ATNT Laboratories, and in multiple European and Asian countries. Soon, production of humanized JAX mice will begin in Australia, eliminating the need to meet current requirements for long quarantine periods.



Left. Genetically-humanized FcRn mice provide translational pharmacokinetics (PK) and pharmacodynamics (PD) data for antibody-based therapeutics. The image illustrates a therapeutic antibody (blue) adhering to its target cell surface.
The Jackson Laboratory

A growing number of mouse and rat models is available for the study of Parkinson's disease through Taconic Biosciences' fifteen-year partnership with the Michael J. Fox Foundation.

Enhancing precision and flexibility

Taconic is also responding to the growing demand for scientific accuracy, customization, and immunology-ready platforms by focusing on enhancing precision and flexibility in preclinical research.

The FcResolv® NOG portfolio lack FcyRs, which reduces murine immune cell interactions with antibody-based drugs. In addition, Taconic's huSelect™ Custom Immune Cell Engraftment Services, a suite of custom humanization capabilities, are suitable for use with any of their CD34+ humanized immune system models to reduce donor-to-donor variability.

"To provide more effective translational tools in oncology, immuno-oncology, and neurodegenerative research, new offerings will include advanced flow cytometry panels and additional Parkinson's disease models," said Mike Garrett, CEO of **Taconic Biosciences.**

The flow cytometry panels were developed to provide reproducible, high-content immune data in order to streamline study design and support deeper and more standardized immune profiling. The panels are specifically optimized for Taconic's humanized immune system mice models to characterize key immune cell subsets—including T cells, B cells, NK cells, and myeloid populations—across blood, spleen, and bone marrow.

"Our 15-year partnership with The Michael J. Fox Foundation is making available a growing number of mouse and rat models for the study of Parkinson's disease," added Garrett. "New models include the aSyn KI/KO Rat, PINK1KO Rat, and the LRRK2 KO Rat."

A strategic shift in several integrated services should enhance early-stage drug development. Transgene characterization enables rapid, in-depth validation of gene insertion and expression in custom genetically engineered models, while CRISPR off-target analysis helps detect unintended genome edits.

Tiered Custom Model Generation Solutions complement these tools, offering a variety of advanced model generation services tailored to project timelines, budget, and health standards requirements.

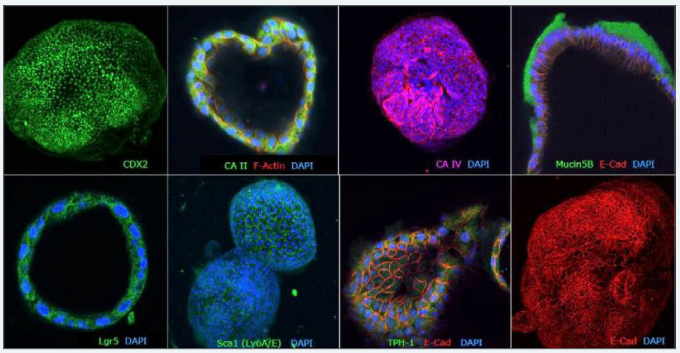
"To support customers, we are also investing in comprehensive phenotypic datasets and partnerships to co-publish preclinical findings and positioning our models to slot seamlessly into tiered workflows integrating AI, animal alternatives, organoids, and animal models," highlighted Garrett.

Lastly, a new CRO preclinic partnership

MilliporeSigma Integrates HUB

The acquisition of HUB Organoids reflects the strategy of MilliporeSigma, the U.S. and **Canada Life Science** business of Merck KGaA, Darmstadt, Germany, to enable wider access to HUB's technology for more effective, faster, and more ethical drug development.

"HUB's intellectual property and service expertise around organoids further strengthens our robust portfolio in 3D cell culture and next-generation biology," said Heather Hargett, PhD, head of Cell Biology Reagents & Tools at MilliporeSigma. "We are committed to our existing partners and look forward to enabling faster market adoption by delivering cutting-edge capabilities to advance the field of next-generation biology."



Immunocytochemical characterization of human colon organoids (Colon-87, SCC321). Human colon patient-derived organoids are positive for colon-specific markers CA II, CA IV, and Mucin5B, the posterior hindgut marker CDX2, stem cell markers Lgr5 and Sca1, and epithelial markers TPH-1 and E-Cad (SigmaAldrich.com/intestinalPDObiobank). MilliporeSigma

The promise of organoids aligns with regulatory agency advocacy on the use of NAM and directly contributes to MilliporeSigma's sustainability ambitions. The use of organoids may allow researchers to limit their reliance on animal testing by reducing stages in the R&D process—as outlined in the FDA Modernization Act 2.0/3.0 and Directive 2010/63/EU—thus positively contributing to more environmentally sustainable alternatives.

Organoids also create opportunities for genetically diverse populations to be reflected in research to allow scientists to better understand drug interactions in patient populations that can be underrepresented in clinical trials.

"As a global company with operations around the world, our top priority remains ensuring that patients, researchers, and customers worldwide continue to benefit from our innovations without disruption," emphasized Hargett.

The State of Multiomics & NGS

WATCH NOW ON DEMAND

2025 marks the 25th anniversary of the first declaration of the Human Genome Project. The 'omics' field has advanced spectacularly since then, fueled by the advent of next-generation sequencing (NGS) and related technologies such as proteomics and spatial biology. So where does this leave us?

In *GEN*'s "The State of Multiomics and NGS" virtual summit, we proudly host a magnificent group of talented researchers from industry and academia discussing key topics including spatial proteomics (the *Nature Methods* 2024 'Method of the Year'), single-cell biology, spatial omics, and perspectives on the stunning pace of NGS advancement and progress in genomic medicine. In addition, the summit included breakout sessions from our sponsors and plenty of live audience discussion.

FEATURED SPEAKERS

Clive Brown
formerly Oxford Nanopore

Cecilia Lindskog PhD
Uppsala University

Francis S. Collins MD, PhD
formerly NHGRI, NIH

Sarah Teichmann PhD
Wellcome Sanger Institute

Jacob Thaysen PhD
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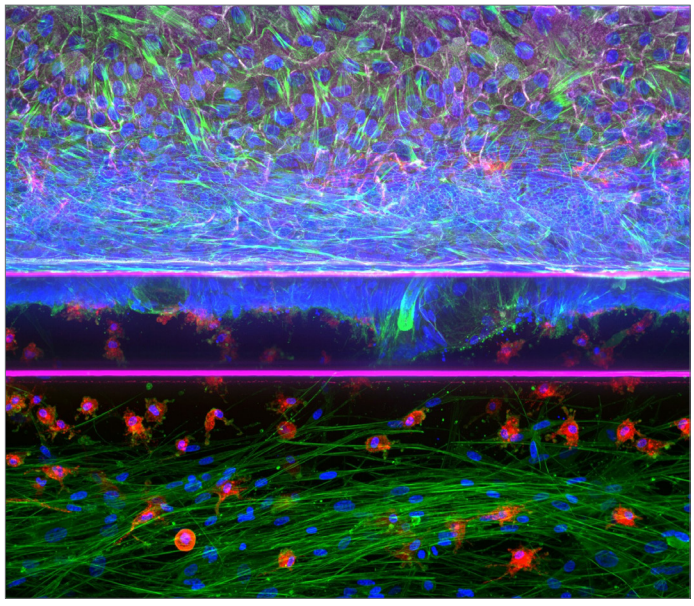
provides customers with greater access to an end-to-end platform by combining the most relevant animal models, custom model generation, and colony management services with downstream *in vivo* study capabilities.

Efficient translational options

The demand for more translational and efficient alternatives to traditional animal models shapes model development. "The need for more predictive preclinical research, particularly in immuno-oncology, infectious diseases, and personalized medicine, continues to drive the advancements in humanized models," said Steve Bronson, DVM, scientific product manager at **Charles River Laboratories**.

The Charles River models NCG-hIL2 and NCG-hIL15 are built on the NCG background, which lacks functional/mature T, B, and NK cells, and has reduced macrophage and dendritic cell function. NCG-hIL2 expresses the human IL2 cytokine, and NCG-hIL15 expresses the human IL15 cytokine. When engrafted with human HSC, both strains support the growth and development of NK and T cells. NCG-hIL15 further supports the homeostatic expansion and survival of NK cells, allowing for stable, long-term immune cell function.

The humanized portfolio will add two PBMC humanized models, HuPBMC NCG-B2m-KO and HuPBMC NCG-MHC-dKO, to support studies of immune responses, tumor growth, and infectious diseases. In addition, an exclusive, do-it-yourself PBMC Select Humanization kit gives researchers more time to create humanized mice with validated PBMCs from recallable donors per their study timeline.



The 3D human gut models from MIMETAS combine colon organoids, fibroblasts, and macrophages, thus providing a physiologically relevant platform to study immune responses, epithelial integrity, and drug efficacy. The image depicts an overlay (*top*) and a 20X view (*bottom*) visualizing actin (*Green*), CD163 (*Red*), and Ezrin (*Pink*). MIMETAS

Bronson added, "More researchers need flexibility in study design with immunodeficient and humanized mice so that they can mimic human immune interactions. By working with these models, researchers can support the 3Rs by lengthening research times and using fewer animals."

Alternative translational models

Microphysiological systems (MPS) address the critical need for better models to understand diseases, find targets, and triage compounds. These technologies fill gaps where current animal models fail, rather than compete head-to-head with them. "The most urgent use for MPS is to meet needs for the development of new molecular entities such as bi- and tri-specific antibodies, antibody-drug conjugates, CAR T cells, and gene therapies, amongst others," said Paul Vulto, PhD, CEO of **MIMETAS**.

MIMETAS's service offering concentrates on the interplay between cell types as primary drivers for disease processes. Their breakthrough liver model, used for assessing gene therapy and toxicity, as well as for fibrosis and MASH modelling, comprises a perfused vascular plexus from liver sinusoidal endothelial cells, stellate cells, hepatocytes, and immune cells. The self-organizing hepatic tissues are indistinguishable from primary liver slices.

The company's CAR T testing services for solid tumors follow the activity of CAR T cells from the bloodstream to the tumor. The tumor comprises critical tumor microenvironment elements, including vasculature, stroma, and immune cells.

UniFlow technology has extended the services portfolio by allowing perfusion of vasculature under unidirectional flow conditions. As such, the technology provides more relevant cues while still retaining the throughput of the original OrganoPlate® technology, and is applied to liver and tumor modelling. "Another new service offering is our lung modelling work for studying diseases such as pulmonary fibrosis and toxicity," said Vulto.

To go further, the new OrganoReady® Colon Organoid plates provide 64 colon tubules based on the HUB organoid technology. "Next in line will be a full launch of our OrganoReady HBMEC plates that allow compound testing on blood-brain barrier transport and toxicity," said Vulto.

Vulto feels that the smooth continuity of the FDA drug approval process is vital, and if flawed during restructuring, it will threaten the entire industry. The market is currently improving now that large pharma restructuring, priority shifts to late-stage development, and redefinition of early-stage R&D focus are complete. Unfortunately, a decline in grant funding, particularly in the U.S., will have yet-to-be-determined negative consequences. GEN

Taconic Biosciences and The Michael J. Fox Foundation Reimagine Preclinical Partnerships to Overcome Parkinson's

Curated portfolio of mouse and rat models advances neurodegenerative disease research

Parkinson's disease (PD) affects millions globally, disrupting motor function and quality of life. While roughly 10% of cases are linked to genetic mutations, the majority result from complex interactions among aging, environmental exposure, traumatic brain injuries, and other risk factors.

Preclinical PD research focuses on identifying early biomarkers to predict disease onset, uncovering strategies to slow or halt its progression, and developing therapies that can alleviate existing motor and non-motor symptoms. By studying the disease in animal models, researchers aim to build a foundation for more effective diagnosis and personalized treatments. However, understanding the intricacies of this complex, multifactorial disease is hampered by the lack of readily available preclinical models that accurately recapitulate disease onset, poor reproducibility among studies, and logistical challenges in accessing reliable models—factors that continue to slow progress in the field.

Taconic Biosciences

Developing strategic solutions

To overcome significant gaps in the scientific toolkit for Parkinson's disease (PD) research, Taconic Biosciences partnered with The Michael J. Fox Foundation for Parkinson's Research (MJFF) to provide broad access to a comprehensive portfolio of genetically engineered mouse and rat models that replicate key features of the disease. Murine models are indispensable in PD research, as they mimic the degeneration of dopamine-producing neurons and enable the study of genetic contributors, such as *PRKN* and *SNCA* mutations, which are critical to understanding disease onset and progression.

This collaboration has expanded the availability of translationally relevant tools, including the floxed parkin mouse, which features a conditional *Prkn* allele in which exon 7 is floxed (linked to early-onset PD), and the humanized aSyn A53T *SNCA* knock-in rat, which expresses a pathogenic alpha-synuclein variant while lacking endogenous rat *SNCA* function. These models, along with the newly introduced *SNCA* knockout rat and the constitutive knock-in LRRK2 mouse with a human point mutation, along with nearly a dozen additional models, allow researchers to investigate hallmark disease processes like alpha-synuclein aggregation and Lewy body formation. With five more models in development, the portfolio is helping to overcome barriers such as limited access, poor reproducibility, and inadequate disease representation, ultimately advancing the discovery of tailored therapies and bringing the field closer to disease-modifying treatments and a cure.

15 years of partnership

In addition to equipping researchers with a diverse portfolio of genetically targeted mouse and rat models, this partnership between MJFF and Taconic Biosciences offers streamlined, up-front licensing terms that clearly define research use, breeding rights, and distribution permissions—removing ambiguity and accelerating scientific progress. By minimizing administrative and logistical hurdles, the collaboration empowers researchers to focus on uncovering the mechanisms of Parkinson's disease and developing effective therapies. Many investigators also choose to leverage Taconic's expertise in breeding and colony management, freeing up valuable time and resources to concentrate on experimental design and data analysis.

This long-standing partnership, established in 2010 as part of MJFF's Research Tools Program, reflects a shared commitment to advancing the field by providing broad, equitable access to rigorously characterized, high-quality rodent models. These models, essential for probing disease mechanisms and testing interventions, are a cornerstone of MJFF's mission to find a cure and improve the lives of those living with PD today.

"The Michael J. Fox Foundation is dedicated to finding a cure for Parkinson's disease through an aggressively funded strategic research agenda and to ensuring the development of improved therapies for those living with Parkinson's today," said Nicole Polinski, PhD, director of research resources, MJFF.

The tools generated through this collaboration represent a critical step toward that goal. Researchers can explore the full range of available PD models at taconic.com/mjff. ■

Biology's Bottleneck: AI Can't Deliver Without Better Lab Infrastructure

By Jon Brennan-Badal

Traditional laboratory processes need to change to take full advantage of the power of AI

John Brennan-Badal
CEO, Opentrons

We are in an odd moment: AI systems trained on roughly 20 trillion text tokens stand ready, yet biological labs still rely on hand-held pipettes and improvised spreadsheets.

Even after revolutionizing fields like transportation and search, AI's transformative potential in life sciences remains handcuffed by deeply entrenched, manual, and inconsistent experimental routines. These limitations are compounded by a fragmented ecosystem—one characterized by incompatible data formats, insufficient data sharing across institutions, and challenges in gathering large, high-quality multimodal datasets at the necessary scale and consistency.

In addition, unlike fields with relatively well-defined outputs, life sciences demand different types of AI tools: from predictive models for protein folding and therapeutic binder design with minimal toxicity, to causal AI frameworks that map complex biological networks directly to clinical outcomes.

This imbalance limits discovery long before GPUs enter the picture. Academic and industrial teams face thin, siloed data streams, not computer shortages. The fix starts by re-engineering the physical lab so it speaks natively to computation.

The cost-curve lesson from genomics

In 2001, sequencing a single human genome cost about $100 million; relentless miniaturization and sensor advances have since driven that price below $100. Genomics did not accelerate because algorithms outran Moore's Law, the price per base read collapsed. Small-molecule screening and cellular phenotyping have not yet experienced a comparable cost curve. Until assays become as cheap and routine as next-generation sequencing, biological AI will remain data-starved.

Our route forward combines aggressive automation, modular infrastructure, open protocols, and natural-language interfaces that let scientists steer robots as easily as they now query chatbots. Think of a desktop robot that feeds data to biology the way GPUs feed tokens to language models.

Optimizing workflows through automation

Modern automation can cut reagent and labor costs by 95% or more, converting experiments that once required days of hands-on work into continuously running data lines. Robots paired with programmable workflows allow a single researcher to launch thousands of assays in parallel, eliminating pipetting variance and incubation drift. Every run automatically writes a machine-readable log into analytics pipelines—no manual transcription required. Scientists move from repetitive chores to framing the next hypothesis.

A manual workflow might yield thousands of measurements; an automated line routinely produces millions—the volume modern models need. Generate a hundred-fold larger dataset and models improve; hit a thousand-fold and entirely new insights emerge.

Time-series cell data at a fraction of past budgets

Pairing automated incubation with label-free microscopy now allows plates to be photographed throughout dose–response experiments. Continuous, time-series cell data, once prohibitively expensive, can be gathered on a single robotic stage for a fraction of historical cost, flagging adverse effects earlier and potentially reducing reliance on animal studies.

Modular platforms for scalable data generation

Modular systems weave robotics, on-deck analytics, and scheduling software so protocols can switch from binder selection to secretion validation with one script, enabling platforms to toggle between yeast display for binder selection and secretion for downstream validation. Standardized software makes vendor-specific differences invisible, allowing research groups to run diverse assays without the need for custom drivers.

When targets change, the same hardware adapts, keeping projects moving and budgets intact.

Large language models become lab co-scientists

Natural-language agents now front lab control, acting as "co-scientists" rather than passive schedulers. Imagine telling an LLM to "screen these sixty thousand compounds at five concentrations," and having the agent translate that intent into machine code, dispatching robots and imagers. If early data reveal an off-target hit, the agent suggests a follow-up plate before the first batch finishes. Experiment design, execution, and analysis merge into a conversation measured in hours.

Open-source science scales trust and reproducibility

Closed automation buries methods behind NDAs. Open protocols in Python run identically in Boston, Bangalore, or Berlin. Labs remix each other's code like developers fork GitHub projects, converging on best practice faster. Open hardware and software put high-throughput lines within reach of small startups and academic cores, widening the community that returns data to the commons.

Aligning capital with cost per datapoint

Funding should flow first to assay throughput and then to intricate network design. Lowering the marginal cost per datapoint, rather than inflating model complexity, delivers the greatest lift in predictive accuracy once datasets grow past one million interactions.

A new model for scientific discovery

Self-driving cars learn from billions of road-miles, yet biological models train on datasets counted in the low millions. Producing a billion-row protein-binding dataset with conventional methods can cost about a billion dollars. Miniaturized automation can drop that figure to roughly ten million, turning a fantasy into a realistic consortium project.

Scale alone is not enough. Silos, inconsistent formats, and one-off experiments still blunt progress. What we need are structured, traceable, continuously refreshed evidence streams that AI can use immediately.

By replacing project-based assays with automated, always-on pipelines, we generate those streams. Complex cell screens that once ran only at large pharmas, now execute overnight on modular robots. Abundant, standardized data lets AI move from suggesting drug candidates to computationally confirming binding, toxicity, and metabolic fit before a chemist lifts a beaker.

Envision the clinic-to-cure loop

AlphaFold solved structure; a multi-billion-row binding dataset will solve interaction. Combine those two, and the clinic experience changes entirely.

Picture a future clinic visit where you give a blood sample and walk out with a molecule tailored to your specific biological receptors, because an AI already validated binding, toxicity, and pharmacokinetics against a vast, low-cost dataset. Personalized therapy in days rather than years is just one of the benefits that becomes reality once the lab's data drought ends.

The mandate is plain: automate aggressively, share openly, and measure progress by cost per datapoint. Do that, and data-based knowledge, not imagination, will power the next era of discovery. GEN

Jon Brennan-Badal is CEO of Opentrons.

Pairing robots with programmable workflows can empower a single researcher to do thousands of assays in parallel.

Editor's Choice

Scientists Are Turning Back the Clock

Regenerative medicine seeks to repair, replace, or regenerate aging cells and tissues

By Kathy Liszewski

Greenland sharks can live up to 500 years, while adult mayflies are fortunate to exist for just a day. Although our cellular clocks differ dramatically, scientists argue that human aging is one of the highest risk factors for a host of degenerative and deadly conditions. To better understand, control, and even reverse the aging process, researchers are exploring the promise of regenerative medicine—a groundbreaking field that focuses on repairing or replacing damaged cells, tissues, and organs to restore normal function.

GEN spoke to several leaders in the field about their regenerative medicine programs and the applications they are pursuing. One approach utilizes the massive computing power of AI to identify safe drug combinations that can simultaneously target and treat multiple age-related biological pathways. Cells can be rejuvenated by harnessing the directorial capabilities (on/off switch) of epigenetics with engineered adeno-associated viruses (AAV) carrying transcription factors. An implantable bio-hybrid organ bearing healthy human pancreatic cells has shown promise in correcting type 1 diabetes (T1D).

Above. Parkinson's disease and other degenerative illnesses feature progressive dysfunction that can lead to cell death. BlueRock Therapeutics is developing regenerative pluripotent stem cells to replace damaged cells and restore function.

Parkinson's disease may be treatable by transplanting precursor stem cells that can differentiate into function-restoring mature cells. While suitable aging models are still a challenge, one model system force-ages stem cells in a dish, couples this with CRISPR screenings, and has shown promise in identifying rejuvenation genes.

Targeting multiple pathways

"Age-related diseases are driven by interconnected biological changes that evolve with age, described as the hallmarks of aging," explains Ann Beliën, PhD, founder and CEO of **Rejuvenate Biomed**. She continues, "Understanding this complexity is crucial for the development of targeted interventions that can effectively change the course of disease and enhance the quality of life for the aging population."

One therapeutic challenge is that single-target drugs often do not fully address such complex multifactorial processes. According to Beliën, the company is addressing this need by pursuing synergistic combinations of therapeutics that can impact multiple pathways simultaneously to enhance clinical benefit. "We are using drug combinations with established safety profiles for derisking clinical development and shortening the development time. This approach thus presents an opportunity to address medical unmet needs in severe diseases of aging."

The company identifies and assesses the combos using two synergistic platforms: the AI-enabled CombinAge™ and *in vivo* CelegAge™ platforms. "These enable a biology-first and disease-agnostic approach," informs Beliën.

The CombinAge platform sifts through vast datasets of biomedical knowledge on hundreds of individual drugs already proven safe for older adults and "analyzes pathways linked to hallmarks of aging, such as mitochondrial dysfunction, epigenetic alterations, and intercellular communication, to uncover combinations of those individual drugs that can impact multiple aging pathways simultaneously."

After an additional safety check on the identified drug combination, its impact on health span is assessed *in vivo* via CelegAge, a platform that employs *C. elegans* worms. Beliën summarizes, "This model focuses on outcomes like movement, speed, and overall mobility, key indicators of aging and vitality, to validate CombinAge's predictions."

Following these analyses, the company goes back to CombinAge, which contains disease-specific data, to predict which age-related disease the drug combination could most effectively treat. The combination is then ready for the typical preclinical process and suitable to be advanced in Phase II trials directly, after the proof of principle is demonstrated in animal models.

The company has built a pipeline of five unique combination drugs targeting neuromuscular, musculoskeletal, metabolic, cardiovascular, nephrological, and neurodegenerative diseases. Beliën asserts that its lead Phase II-ready asset, RJx-01, has already demonstrated significant potential in treating sarcopenia. "We look forward to initiating a Phase II trial to evaluate sarcopenia in patients with chronic obstructive pulmonary disease this year."

Epigenetic focus

Aging has historically been viewed to result from random wear and tear on the body that accumulates throughout a person's life. **Life Biosciences** says that these biological aging processes are modifiable. Sharon Rosenzweig-Lipson, PhD, CSO, provides a perspective: "As we age, the epigenetic code that regulates gene expression drifts, leading to altered patterns of gene expression. This shift is associated with changes in epigenetic markers called methyl groups that may lead to dysfunction."

Ann Beliën, PhD
Founder, CEO
Rejuvenate Biomed

Sharon Rosenzweig-Lipson, PhD
CSO, Life Biosciences

Scientists at Rejuvenate Biomed utilize AI to identify synergistic drug combinations that can target multiple pathways simultaneously for the treatment of age-related diseases.

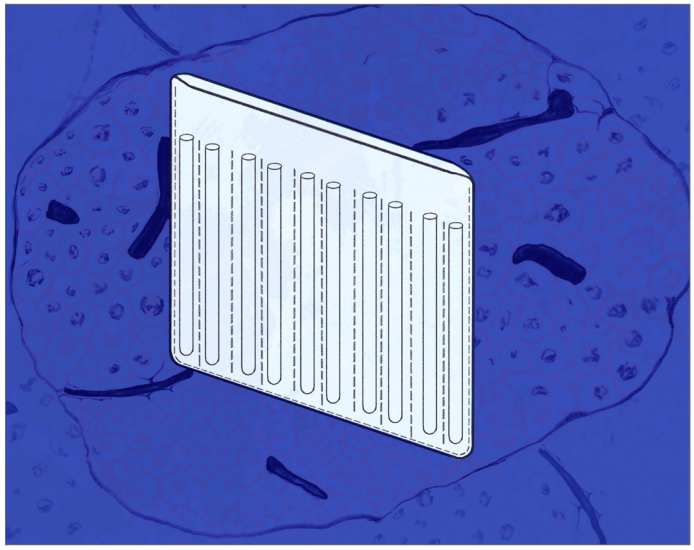
The company is focusing on partial epigenetic programming based on the Nobel-Prize-winning discovery of Yamanaka factors. These four transcription factors can completely dial back and reprogram mature cells to become pluripotent. Rosenzweig-Lipson explains, "Life Bio is leveraging our partial epigenetic reprogramming platform using three factors, OSK (Oct4, Sox2, Klf4), to reset the epigenetic code and reverse age-related epigenetic changes. This allows for cellular rejuvenation to a younger state, without the loss of cell identity, to prevent or reverse age-related diseases. This approach targets a key root cause of aging at the epigenetic level, thereby offering the potential to address a wide range of age-related diseases with significant unmet medical needs."

Life Biosciences' lead candidate is ER-100. Rosenzweig-Lipson elaborates, "ER-100 utilizes a dual vector AAV2 tet-on system containing an AAV2 with a transactivator and an AAV2 that can express OSK in the presence of oral doxycycline. This permits the expression of OSK within target cells to reprogram the epigenome in a controlled manner."

ER-100 is currently in late-stage preclinical development for both chronic and acute optic neuropathies including glaucoma and the rare eye disease non-arteritic anterior ischemic optic neuropathy (NAION). Rosenzweig-Lipson reveals, "ER-100 has demonstrated safety and efficacy in multiple pre-clinical animal models of disease. We are aiming to initiate the first human clinical studies evaluating ER-100 for optic neuropathies within a year."

Bio-hybrid organ

Regenerative medicine also provides a promising avenue for treating chronic diseases such as T1D, an autoimmune disorder that destroys the insulin-producing cells of the pancreas. **Sernova** is focusing on restoring the body's natural ability to regulate insulin by combining its implantable Cell Pouch™ bio-hybrid organ with therapeutic human cells.



One of the hallmarks of T1D is destruction of insulin-producing pancreatic cells. Sernova is pioneering the use of an implantable Cell Pouch that houses healthy donor islet cells to create a bio-hybrid organ.

CEO Jonathan Rigby explains, "In the case of T1D, the therapeutic cells that are transplanted into the Cell Pouch chambers are pancreatic islets. Islets are clusters of alpha, beta, and delta cells, which create vital hormones called glucagon, insulin, and somatostatin. These hormones regulate blood glucose. By transplanting islets into the chambers of the Cell Pouch bio-hybrid organ, we aim to provide a location within the body for the long-term survival and function of the therapeutic cells."

According to Rigby, the Cell Pouch is a small, chambered structure made from nontoxic, medical-grade, biocompatible materials previously approved by the FDA for permanent use in the body. "It is surgically implanted under the skin against the abdominal muscles, where it seamlessly integrates within the body to create an ideal vascularized tissue environment. The cells are transplanted into chambers of the Cell Pouch bio-hybrid organ."

Rigby says the company is currently conducting Phase I/II clinical trials in combination with human donor islets. "The Cell Pouch has shown the ability to support the survival and function of transplanted islets for over five years. In addition, it has demonstrated complete retrievability with no evidence of fibrosis, while also showing promising signs of efficacy."

However, Rigby believes that utilizing donor islets is not a commercially viable option for the future. He reports, "We have partnered with Evotec, a company that has developed induced pluripotent stem cell (iPSC)-derived islet-like clusters, which can be manufactured in nearly unlimited quantities. Notably, in preclinical studies, Evotec's islet-like clusters have performed comparably to human islets when implanted within the chambers of a pre-vascularized Cell Pouch."

Replace-restore-reimagine

In degenerative illnesses, such as Parkinson's disease, cells undergo progressive deterioration and dysfunction, potentially leading to cell death. **BlueRock Therapeutics** is focusing on regenerative pluripotent stem cells (PSCs) that can differentiate into specific cell types that have the potential to replace the cells lost or damaged by disease.

Amit Rakhit, MD, chief medical officer, discusses, "Typically, by the time a person is diagnosed with Parkinson's disease, approximately 80% of their dopamine-producing neurons are gone. This impacts a person's cognitive, motor, and non-motor functions. Current standard of care, using small molecules or deep brain stimulation, can help mimic this loss of dopamine and pro-

vide symptomatic relief, but over time, their effectiveness declines."

According to Rakhit, BlueRock Therapeutics is taking a different approach. They aim to replace the dopamine-producing neurons that are lost in Parkinson's disease by administering PSCs differentiated into dopamine-producing neuron progenitors. "In a one-time surgical procedure, the dopamine-producing neuronal precursors are transplanted into a part of the brain called the putamen. When transplanted, these precursors have the potential to halt disease progression, by further developing into mature brain cells after implantation and reforming the neural networks that have been severely affected by Parkinson's–restoring motor and non-motor function to patients."

Last year, the company's candidate therapy, named bemdaneprocel, completed an open-label Phase I clinical trial in 12 participants. Rakhit reports, "Based on the data from this trial and conversations with the FDA under a Regenerative Medicine Advanced Therapy designation, BlueRock plans to advance bemdaneprocel to a Phase III pivotal trial that is expected to start later this year. This will be the first registrational Phase III clinical trial for an investigational allogeneic stem cell-derived therapy for treating Parkinson's disease."

> **"As we age, the epigenetic code that regulates gene expression drifts"**
>
> —Sharon Rosenzweig-Lipson, PhD
> CSO, Life Biosciences

BlueRock also plans to initiate a Phase I clinical trial for an investigational iPSC-derived therapy, OpCT-001, for the treatment of primary photoreceptor diseases that cause irreversible vision loss in children and adults.

Rakhit believes that such allogeneic cell therapies offer the potential of achieving a global impact. "We look forward to continuing to work with our colleagues in cell therapy to advance the field and bring these cutting-edge treatment options forward, and aspire to transform medical practice of the future."

Aging in a dish

Koby Baranes, PhD, head of science at **Clock Bio**, cautions, "Aging is not yet fully understood and existing models often fail to fully replicate the complexity of human aging, making it difficult to conduct high-throughput screening and accurately predict the efficacy and safety of potential interventions."

Thus, the company is working to "reset the clock" by utilizing a stem cell model. Baranes elaborates, "We have developed a proprietary aging model–an aging intervention–that enables us to force age human iPSCs. By applying this intervention, we can faithfully recreate all known cellular hallmarks of aging."

Baranes says these iPSCs are unique in that after forced aging, they spontaneously show aging reversal. "iPSCs self-rejuvenate within days, meaning that the cellular hallmarks of aging disappeared. Given that these hallmarks are cellular phenotypes of age-related disease, our hypothesis is that understanding this self-rejuvenation process should yield therapeutically translatable insights."

Company scientists have systematically identified genetic factors involved in this aging process using genome-wide CRISPR screening that selectively knocks out or activates all individual genes across the genome. Baranes reports, "This unbiased approach allows us to determine the role of each gene in rejuvenation. If knocking out a gene impairs rejuvenation, it suggests that the gene is essential for the process. Conversely, if knocking out a gene accelerates rejuvenation, it may function as an aging-promoting factor."

Employing this strategy, the company has identified more than 150 genes so far. "We collectively call this the 'Atlas of Rejuvenation Factors.' Our early findings suggest that approximately 25% of these genes are 'rejuvenation genes' (whose loss inhibits rejuvenation), while around 75% are 'aging genes' (whose loss enhances rejuvenation). Therefore, we have sufficient degrees of freedom to potentially operate with classic modalities of protein inhibition."

Clock Bio scientists have tested more than 300 drugs, with over 100 that target their hits using a proprietary pre-clinical drug discovery assay. Baranes summarizes, "This allows us to validate the effect of our identified genes in somatic cells and prioritize them for clinical application in the near future. We started with validation in fibroblasts and will move to neuronal models and immune cells from there." GEN

Jonathan Rigby
CEO, Sernova

Amit Rakhit, MD
Chief Medical Officer
BlueRock Therapeutics

Koby Baranes, PhD
Science Head
Clock Bio

Genome Editing

Advancing CRISPR Cures Requires Cross-Sector Collaboration

By Fay Lin, PhD

Amid a challenging gene editing climate, partnerships between researchers, regulators, and industry remain crucial for pushing CRISPR cures to the clinic

"We need a patient-first approach for any variant in any patient, whomever, wherever they are. Each and every patient deserves a fair shot at this!" exclaimed Kiran Musunuru, MD, PhD, as he held the crowd captive at the American Society of Gene & Cell Therapy (ASGCT) annual meeting in New Orleans last month.

Musunuru had just announced that the world's first patient was treated with a personalized CRISPR gene editing therapy at Children's Hospital of Philadelphia (CHOP).

The infant, named KJ, was diagnosed with a rare metabolic disease known as severe carbamoyl phosphate synthetase 1 (CPS1) deficiency, an autosomal recessive disorder which causes toxic ammonia buildup in the blood. KJ was too young and vulnerable to receive a liver transplant, the only standard-of-care option for the disease, and with each day that passed, the episodes of increased ammonia put him at risk for ongoing neurological damage or death.

Musunuru, a cardiologist and professor for translational research at University of Pennsylvania School of Medicine, teamed up with Rebecca Ahrens-Nicklas, MD, PhD, assistant professor of Pediatrics at the University of Pennsylvania, regulators, and industry partners to manufacture a personalized base editing therapy designed to correct KJ's individual mutation. The large collaborative effort led KJ to receive the first dose within a swift seven months.

Within seven weeks after infusion day, KJ was able to tolerate increased dietary protein and receive half the starting dose of his nitrogen-scavenger medication, putting him on a promising path of improvement.

While Ahrens-Nicklas emphasized that it is still early days to determine KJ's long-term outlook, she highlights that he has made slow but steady progress. KJ is "safer than he was before" with the research team having "much more to learn from him."

"We want each and every patient to have the potential to experience the same results we saw in this first patient," said Musunuru. "The promise of gene therapy that we've heard about for decades is coming to fruition, and it's going to utterly transform the way we approach medicine."

CRISPR challenges

Nobel laureate and CRISPR pioneer, Jennifer Doudna, PhD, aspires for "CRISPR to become the standard of medical care for certain diseases" when asked for her dream for the technology in the next 5–10 years. That vision has slowly started to come to life.

KJ's case follows the clinical success of Casgevy, a one-time gene therapy for treating sickle cell disease (SCD) and transfusion-dependent beta thalassemia and the first CRISPR-based therapy approved by the FDA. The drug was a collaborative effort between **Vertex Pharmaceuticals** and **CRISPR Therapeutics** and gave new hope to SCD patients who previously had no cure.

Additionally, many experts have eyed promising results from **Intellia Therapeutics** as the next contender for clinical approval. The Cambridge-based company's pipeline includes demonstration of the first *in vivo* gene editing in humans for transthyretin (ATTR) amyloidosis with cardiomyopathy, a progressive disease characterized by buildup of protein fibrils in the heart. The first patient was dosed in a Phase III study last year.

Despite CRISPR's promise, gene editing companies have found themselves in a challenging climate. Earlier this Spring, Peter Marks, MD, PhD, a key ally to the rare disease community who oversaw the approval of 22 gene therapies, resigned from his role as Director of the FDA's Center for Biologics Evaluation and Research (CBER). His departure has left cell and gene therapy's regulatory outlook uncertain.

Left. Baby KJ, the first patient to be treated with a personalized CRISPR therapy, reaches out post-infusion.
Children's Hospital of Philadelphia

Jennifer Doudna, PhD, giving opening remarks at CRISPR Cures Day. Glenn Ramit

Additionally, many gene editing start-ups are facing funding challenges leading to reduced staff and trimmed pipelines. In a bleak announcement last December, **Editas Medicine**, one of the first CRISPR companies founded in 2013, cut 65% of its employees. Additional companies followed suit, including **Intellia**, **Aera Therapeutics**, and **Caribou Biosciences** undergoing layoffs impacting over a quarter of their headcount.

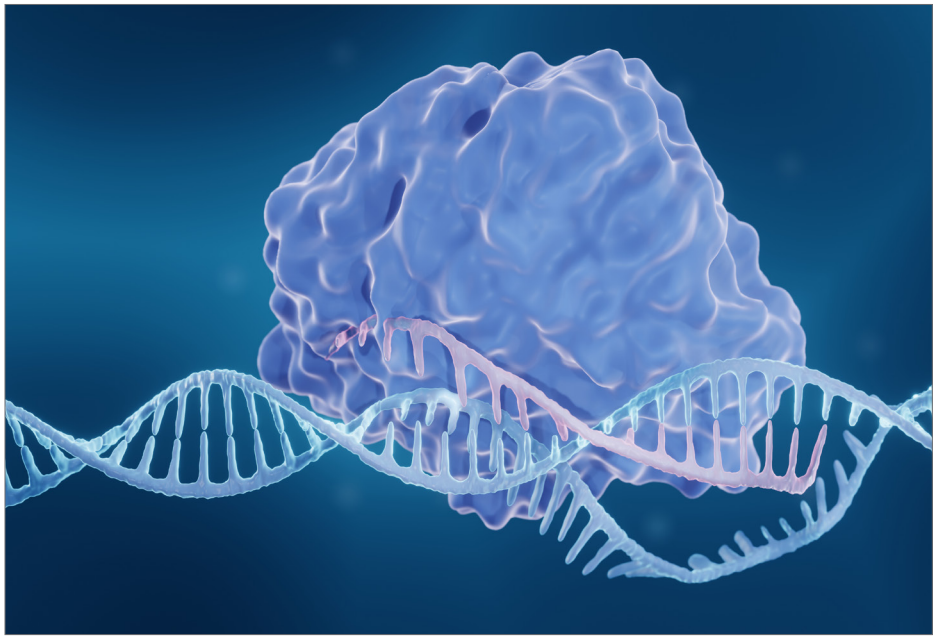
Tome Biosciences, a once well-backed gene editing start-up that launched with $213 million in 2023, folded completely last year. Founded by Massachusetts Institute of Technology (MIT) duo, Omar Abudayyeh, PhD, and Jonathan Gootenberg, PhD, Tome's technology sought to insert large DNA sequences into the genome, a proposed advance over most CRISPR methods which were limited to small edits.

Despite these trends, Benjamin Oakes, PhD, CEO of **Scribe Therapeutics**, says genetic medicine companies have comparatively held up better over a biotech downtown that's "blown out the whole sector" for almost four years.

"The challenge comes from the broader market, but in reality, you're seeing nothing but good data come out of the CRISPR space," Oakes told *GEN*. "I don't believe the ability to cure patients is going anywhere. We are all resistant to change, especially if it's a change to the medical paradigm."

Scribe is a Bay Area-based CRISPR gene editing company co-founded by Doudna. As natural CRISPR systems originate from bacteria, Scribe's platform features a new CRISPR enzyme, termed CasX, which is engineered to improve the potency, specificity, and safety of gene editing in humans. Additionally, Scribe's epigenetic silencing platform, termed Epigenetic Long-term X-Repressor (ELXR), turns off gene expression of multiple targets without cutting DNA.

Fyodor Urnov, PhD, director of technology and translation at the Innovative Genomics Institute (IGI), who recently authored a 6,000-word editorial in *The CRISPR Journal* to "give Cas a chance," says there's no magic want that enables a technology to change the world. Experiencing resistance when on route to public acceptance is not uncommon for a new therapeutic modality.



CRISPR-Cas9 genome editing enzyme. Artur Plawgo / iStock / Getty Images Plus

"CRISPR fundamentally can do what we wanted to do, but a good chunk of the regulatory environment, for example, putting CRISPR into a person, hasn't changed in 15 years," Urnov told me during my recent visit to the IGI earlier this Spring.

Amber Salzman, PhD, CEO of **Epicrispr Biotechnologies**, sees the shift as a more selective market, with investor interest in CRISPR and gene regulation remaining strong, especially for platforms with "differentiated science and clear clinical potential."

"What we're seeing isn't a pullback, but a shift in focus. Investors are now looking for companies that can bridge from breakthrough science to real-world impact for companies with compelling data, well-defined pipelines, and a clear regulatory and commercial strategy," Salzman told *GEN*.

Partner up

As a new therapeutic modality, CRISPR therapy must establish its own research, manufacturing, regulatory, and commercial pipelines to achieve societal uptake, with delivery being one of the biggest barriers to the clinic.

To receive Casgevy's *ex vivo* therapy, patients must undergo a bone marrow transplant, a brutal approach that is also expensive and difficult to scale. Additionally, KJ's in vivo personalized CSP1 treatment targeted the liver, where the delivery challenge has largely been solved. Clinical proof points showing successful *in vivo* targeting to broader tissues have yet to come through the gate.

New early gene editing platforms are rising to address the *in vivo* challenge. In May, **Stylus Medicine**, co-founded by Patrick Hsu, PhD, core investigator at the Arc Institute, launched with $85 million to advance precise, durable CAR T generation inside the body. The company's approach uses cell-targeted lipid nanopar-

ticles (LNPs) to deliver therapeutic payloads directly to immune cells *in vivo*.

On the commercial side, CRISPR's proposed paradigm shift to cure patients with a single treatment has historically left investors scratching their heads. Even if a new therapeutic modality "looks good in a lot of ways," there remains uncertainty about commercial adoption, commented Erik Sontheimer, PhD, professor at the RNA Therapeutics Institute at UMass Chan Medical School in an interview with *GEN*.

To address these challenges, Sontheimer says collaboration with companies that have strong regulatory and commercial experience, in addition to a compatible approach and culture, goes a long way toward supporting long-term clinical success. He noted that synergistic pairings, such as CRISPR Therapeutics and Vertex, and Intellia and Regeneron, largely contributed to the success of Casgevy and the ATTR therapeutic program, respectively.

To support this mission, **Danaher** and the IGI launched the Danaher-IGI Beacon for CRISPR Cures program in early 2024 to produce a "stacked" manufacturing platform for gene editing therapies with the goal of increasing the number of patients treated with CRISPR-based technology from approximately 210 patients today to many thousands over the next decade.

In this collaboration, Danaher provides manufacturing and technological support to CRISPR therapy researchers within the IGI ecosystem by leveraging its more than 15 diverse businesses, including **Integrated DNA Technologies** (**IDT**), **Cytiva**, **Molecular Devices**, **Leica**, **Beckman Coulter**, and **Aldevron**.

This vision has translated into impact. Aldevron and IDT were among the crucial team that effectively manufactured KJ's historic personalized CRISPR gene editing drug, which required a new guide RNA (gRNA) sequence, mRNA-encoded base editor, custom off-target safety services, and clinically validated lipid nanoparticle (LNP) formulation, all on a swift time scale of a few months.

"Our mission in academic science is to create new things and explore new ideas. What we're not good at is developing ideas so that they can be widely available, affordable, and disseminated," said Doudna at CRISPR Cures Day, a celebration of the collaboration's one year anniversary hosted at the IGI in February. "This partnership is our effort to bring those two areas together so that CRISPR can have a global impact."

While CRISPR is an "electric car in a universe that was built for early model internal combustion engines," to use Urnov's words, the promise of gene editing technology remains at the forefront. Time will tell how the power of collaborative minds will push the next wave of CRISPR cures to the clinic. GEN

A-List Continued from page 15

preclinical proof of concept for functional upregulation of an undisclosed target liver protein to reduce a disease biomarker by >80%. With $4.658 million in Q1 collaboration and R&D revenue, Editas is tied for nine in clinical programs (none), but places sixth in cash position ($220.964 million) and seventh in market cap ($115.524 million).

7. Caribou Biosciences (NASDAQ: CRBU)

Caribou Biosciences expects to report early clinical data during 2H 2025 on its co-lead candidates CB-010 for large B-cell lymphoma and CB-011 for multiple myeloma. The company narrowed its pipeline to those programs in a "prioritization" that included chopping its workforce approximately 32% (47 jobs) and ending a Phase I trial (NCT06752876) of CB-010 for lupus, a Phase I trial of CB-012 in relapsed or refractory acute myeloid leukemia, and all preclinical research. Generating $2.353 million in Q1 revenue, licensing and collaborations, Caribou placed seventh in cash position ($212.452 million) and clinical pipeline (two Phase I candidates), and ninth in market cap ($76.161 million).

8. Metagenomi (NASDAQ: MGX)

Metagenomi expects to submit an IND and clinical trial application (CTA) in 2026 to advance its first clinical program, wholly owned MGX-001 in hemophilia A, into first-in-human studies. MGX-001 includes a bioengineered Factor VIII (FVIII) construct with higher FVIII activity levels vs. wild type. In May, Metagenomi released 19-month FVIII durability data from a preclinical nonhuman primate durability study showing FVIII levels of 80%, 10% and 32% correlating with gene integration frequency in each of three animals studied (one died prematurely, unrelated to treatment). With $4.127 million in Q1 revenue, all from collaborations, the company finished eighth in cash position ($225.970 million), ninth in clinical pipeline (none), and 10th in market cap ($56.074 million).

9. Prime Medicine (NASDAQ: PRME)

Prime Medicine's new CEO Allan Reine, MD, in May shrunk the company's workforce 25% (about 50 jobs) and pipeline, opting to partner lead clinical candidate PM359 despite positive initial Phase I/II trial (NCT06559176) data in p47phox chronic granulomatous disease. The company will continue its preclinical Wilson's Disease and AATD programs, with INDs and/or CTAs expected in 1H 2026 and mid-2026, respectively. Reine succeeded Keith Gottesdiener, MD, who resigned May 19. Reporting $1.454 million in Q1 collaboration revenue, Prime finished sixth in market cap ($158.866 million) but eighth in clinical pipeline (PRME-359) and ninth in cash position ($144.256 million).

10. Sangamo Therapeutics (NASDAQ: SGMO)

Sangamo Therapeutics said on May 6 that all patients dosed with its Fabry disease gene therapy candidate isaralgagene civaparvovec (ST-920) in the Phase I/II STAAR trial (NCT04046224) passed the one-year milestone under the FDA's Accelerated Approval pathway. In April, Sangamo licensed its neurotropic adeno-associated virus (AAV) capsid STAC-BBB to Eli Lilly under an up-to-$1.4 billion agreement to develop up to five intravenous genomic medicines for central nervous system diseases. With $6.437 million in Q1 revenue, Sangamo is fifth in clinical pipeline (ST-920 and ST-503 for idiopathic small fiber neuropathy, also in Phase I/II) but ranks lower in market cap (eighth with $100.387 million) and cash position (10th with $25.18 million). ■


+OP1

The Final Formulation: Last Step in the Drug Development Journey

Addressing protein aggregation, product stability, supply chains, and regulations requires multiple tools, including predictive computation

By Mike May

All of the basic research and early development behind a biotherapeutic, as well as the preclinical and clinical testing, go for naught without a successful final drug formulation. To benefit patients, all of the research and development must be combined in an actual product. In comparison to a small-molecule therapy, a biotherapeutic final drug formation is more challenging.

According to Daniel Joseph Price, PhD, head of excipients business at the life science business of **Merck KGaA**, Darmstadt, Germany, "Final formulation of biotech drugs and antibodies poses several unique challenges due to the complexity and sensitivity of these macromolecules."

For one thing, the proteins in these biotherapeutics must stay stable. Unfortunately, that's not what proteins tend to do. "Proteins are inherently prone to aggregation, denaturation, and chemical degradation," says Price. "These changes can compromise therapeutic efficacy and safety, particularly for parenteral applications where aggregates can provoke immune responses."

Beyond the natural tendency of proteins to change, the environment can accelerate that problem. "During manufacturing, filling, or transportation, proteins encounter stresses, such as air-liquid interfaces or agitation that can unfold proteins and lead to irreversible aggregation," Price says.

A drug manufacturer can improve protein stability and other features of a biotherapeutic by adding excipients from salts and sugars to preservatives and surfactants. Like the active component in a biotherapeutic, the excipients must be carefully controlled. For example, they "must meet extremely high purity standards due to the high concentrations needed—up to 100 milligrams per milliliter—and the risks associated with injectable routes," Price explains. "Endotoxin load, nanoparticulate impurities, and batch consistency are also critical quality concerns."

Left. To optimize the final formulation for a drug, it must meet many criteria beyond producing a safe and effective product. For example, it must be stable and amenable to various manufacturing steps, such as the automated filling shown here.

Thermo Fisher Scientific

Keeping proteins stable

To stabilize liquid formulations of therapeutic proteins, Merck KGaA optimized its Poloxamer 188 Emprove Expert. "This version of Poloxamer 188 features a high molecular weight and increased hydrophobicity, enabling it to better stabilize proteins against mechanical and interfacial stress," Price says. "In head-to-head forced degradation studies, this product significantly reduced particle formation and protein aggregation across various monoclonal antibodies and fusion proteins, offering performance equal to or better than traditional polysorbate-based formulations."

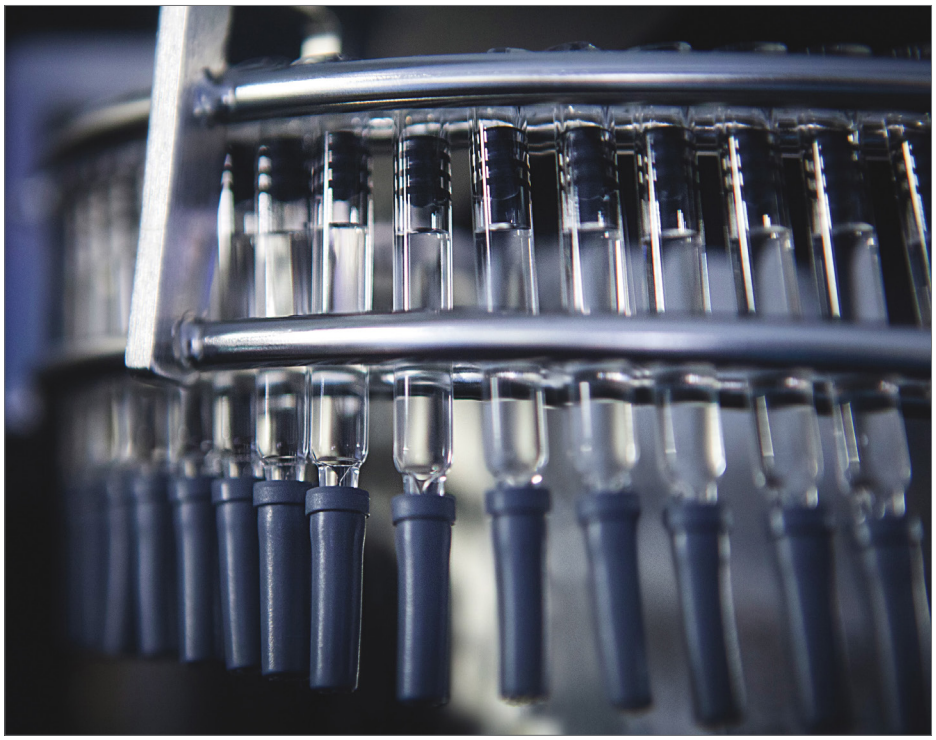
The main challenge in developing Poloxamer 188 Emprove Expert was controlling its complex and heterogeneous

structure. "As a polymeric surfactant, its molecular weight and composition can vary significantly between batches and suppliers, affecting performance," Price says. So, Merck KGaA carefully controlled the material's characteristics, developed a manufacturing process that kept batches consistent, and added 70 parts per million butylated hydroxytoluene as a stabilizer to produce what Price calls a "precision-engineered excipient."

Addressing viscosity in antibodies

Compared to biotherapeutics in general, antibodies pose some unique challenges. During fill and finish, for example, developers must pay special attention to the antibody's "viscosity or levels of excipients used for stability," says Christy Eatmon, global subject matter expert of sterile drug products for pharma services at **Thermo Fisher Scientific**. "As antibodies are concentrated to achieve small injection volumes required for subcutaneous delivery, the viscosity goes up, as well as the propensity for aggregation." A manufacturer of an antibody-based therapeutic must also optimize the level of excipients, such as polysorbate, for stability and minimize the formation of bubbles during filtration and filling. "Another challenge in the filling process is to reduce waste and line losses as much as possible, as these materials are often very expensive," Eatmon says.

Recently, Thermo Fisher introduced excipients or processes that allow for ultra-concentration—above 200 milligrams per milliliter—while keeping the viscosity relatively low, below about 25 centipoise. "The ability to ultra-concentrate will allow for lower dose volumes and the opportunity to deliver these therapies subcutaneously, filling them into a syringe rather than a vial," Eatmon says. "Along with the shift to pre-filled syringes, we are making advancements in the long-acting injectable space, which will reduce dosing frequency and make treatment more convenient for patients."



The formulation of a drug product is developed for a specific application, such as these pre-filled syringes. Thermo Fisher Scientific

Given the large size and complexity of therapeutic antibodies, potential for aggregation and precipitation, and the non-linear viscosity increase as concentration rises, Thermo Fisher keeps many factors in mind during production to develop and manufacture a product that works in a syringe. As Eatmon says, "We need to ensure that the syringeability profile of these molecules is within acceptable limits, as many are used in combination with auto-injectors."

The start impacts the finish

No matter what kind of drug a company is developing, the steps taken at the start play crucial roles in negotiating the entire path to produce a final product. Even when a formulation is being developed from scratch, "you have to make sure that you check all of the regulatory boxes, because there are expectations for specific studies and data," says Travis Webb, CSO at **Pharmaceutics International, Inc** (**Pii**), which works primarily on small-molecule drugs. "That minimizes the number of additional requests that come back from regulators."

In many cases, Pii—recently acquired by **Jabil**, a global expert in engineering, manufacturing, and supply-chain solutions—works on "formulations that other people don't really want to deal with," says Webb. To produce a successful final drug formulation, Pii started using machine learning in the early stages of drug development, such as optimizing a drug's solubility. "So, instead of trying iterative bench trials, we have a standard list of solvents that we use that kind of fill out the 3D space that we map in," he says. In this way, Pii can screen hundreds of solvents or combinations of them in a day. This type of information is used to develop a formulation that can be manu-

factured at a commercial scale.

When asked to assess the value of such modeling, Webb says, "I always make the joke that the best models in the world are 90% correct 80% of the time, and that's not too bad."

Nonetheless, finding the best final formulation for any therapy rarely comes from computation alone. "There's a degree of art that comes with it," Webb says. "The model may tell you something, but based on your knowledge of formulation and formulation components, you can predict how something might vary, especially in scale-up."

Another thing to keep in mind early when developing a drug is what will be in the final formulation and how it will be sourced. "If we have a component come in from a client that's from a single-source provider, we look into that," Webb says. "We may recommend to them that we start doing some work to evaluate a second source because, as COVID taught us, something can happen tomorrow that throws a wrench into everything."

Building in robustness

All manufacturers of biotherapeutics aim to make products more robust to stay safe and effective in patients. To that end, scientists at **Lonza** applied a design of experiments (DoE) approach to assessing the robustness of final drug formulation development.

"One of the key challenges was clearly defining the quality target product profile and selecting relevant formulation parameters that could impact product quality," says Virginie Le Brun, associate director of drug product services formulation development, Lonza. "Identifying these factors early was essential to designing a meaningful robustness study and establishing a reliable design space."

Then, Lonza used a DoE approach to test the composition and concentration of formulation components across a defined design space to assess robustness. "These robustness studies evaluated product stability at the formulation's boundaries, supporting specification settings and ensuring the ability to tolerate minor manufacturing variations—reducing the risk of deviations, delays, or batch rejections," Le Brun says. "This additional knowledge is highly valuable to show robustness in support of the dossier for submission, but also to refine the specifications as well as to understand and support deviations to the target composition during manufacturing."

> **"Final formulation of biotech drugs and antibodies poses several unique challenges due to the complexity and sensitivity of these macromolecules.**
>
> —Daniel Joseph Price, PhD
> Head of Merck Life Science Excipients Business, Merck KGaA

In addition, interactions between a formulation's parameters must be considered to understand the impact of each one on a product's stability. "This can be addressed using a statistical approach," Le Brun says. "Following this strategy, we developed and implemented a statistically optimized DoE designed to characterize the individual effects of formulation variables and their interactions, using a limited number of formulations to reduce material needs and timelines."

For a thorough understanding of a product, though, these tests must also gather long-term stability data at different stability conditions and time points to identify statistically relevant factors, variations, and interactions and their impact on critical quality attributes.

Based on all of this work, Lonza defined "a more reliable and resilient formulation design space, ultimately improving risk management, and supporting regulatory submissions," Le Brun says.

Seeking a more stable future

As biotherapeutics evolve, so too will the steps in final drug formulation. As an example, Price says, "Looking ahead, we aim to expand our portfolio of high-performance stabilizers tailored to next-generation biologics, including bispecifics, fusion proteins, and mRNA delivery systems."

In particular, scientists at Merck KGaA focus on several improvements. For instance, Price says that the company plans to develop multifunctional excipients "that combine stabilizing, buffering, and antioxidant properties." Plus, enhanced analytics will be used "to provide deeper insight into excipient-protein interactions and predict formulation stability early in development," he says.

Beyond testing existing formulations, Merck KGaA also plans to use digital predictive technologies "that can use first principles chemical and biological information to predict optimal formulations of biologics," Price says. "So far, we have focused our digital-information platforms on small-molecule formulation, but we are already starting to develop solutions with our mPredict digital product for large molecules too."

Other companies also envision improved approaches to developing the final formulation of biotherapeutics. "Further optimization of predictive modeling and high-throughput screening tools tailored to the needs and complexity of new molecular formats will be crucial," says Le Brun. "These advancements will help reduce material requirements and accelerate formulation development timelines, benefiting drug developers and their patients." ■

Mass Spectrometry-Based Proteomics: Dead, Dying, or Dark Horse?

MOBILion is advancing MS-based technologies to unlock proteomics' full potential

By Frederick G. Strathmann, PhD, Philip L. Lorenzi, PhD, and Daniel DeBord, PhD

For decades, mass spectrometry has been the gold standard in proteomics because of its combination of specificity, sensitivity, high target plex, and lack of requirement for affinity reagents. Yet, its promise remains largely unrealized outside of specialized research labs. Nowhere is this more evident than in the analysis of the plasma proteome, where mass spectrometry has had to sit on the sidelines, reluctantly watching other, often inferior technologies (based on menu, proteoform specificity, analytical specificity, and cost per target) advance the field toward the long-sought goal of discovering protein-based diagnostic, prognostic, and therapeutic markers.

Whereas genomics and transcriptomics have flourished[1] and integrated seamlessly into clinical and translational workflows, proteomics has struggled to keep pace.[2]

However, with recent innovations in ion utilization, fragmentation strategies, and data acquisition, mass spectrometry stands on the brink of unleashing a new era of proteomics, one where it reclaims its role as the definitive tool for protein-based biomarker discovery, clinical diagnostics, and beyond.

Mass spectrometry has undergone rapid and remarkable advancements over the past several decades, transforming from a niche analytical tool into a powerful engine for proteomic discovery. The evolution began with innovations like electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), which enabled the efficient introduction of biomolecules into the mass spectrometer. Those techniques laid the foundation for modern proteomics by making it possible to analyze intact proteins and peptides with greater sensitivity and resolution.

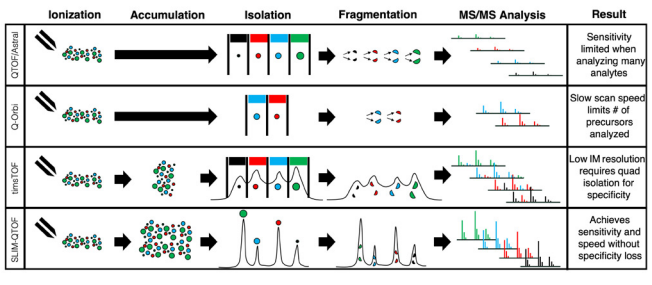


Figure 1. Diagram comparing various methodologies for generating fragmentation spectra.

The introduction of time-of-flight (TOF) and Orbitrap analyzers in the 1990s and early 2000s further enhanced mass accuracy and resolution, enabling high-confidence identification of a greater number of peptides per sample, driving increased demand for proteome profiling. Coupling these high-resolution analyzers with tandem mass spectrometry techniques provided deeper structural insights, while advancements in data acquisition strategies—such as data-independent acquisition (DIA) and parallel accumulation-serial fragmentation (PASEF)—have increased speed, coverage, and specificity.[3]

Most recently, ion mobility spectrometry has been integrated with mass spectrometry to add another dimension of separation, improving sensitivity and decreasing spectral complexity (*see Figure 1*). However, despite these advances, mass spectrometry-based proteomics workflows remain more time-consuming, labor-intensive, and variable (with respect to quantitation) within and across laboratories.

Mass spectrometry's impact across proteomics

Mass spectrometry continues to drive significant advances in life science research and clinical applications. Notable technological improvements have given rise to exciting new areas of research, including:

- **Single-Cell Proteomics:** Mass spectrometry has enabled protein analysis at the single-cell level, providing insights into cellular heterogeneity and protein regulation. Techniques like SCoPE2[4] and scDVP[5] have revolutionized our ability to study individual cells and their dynamic proteomes.
- **Post-Translational Modifications (PTMs):** Mass spectrometry is unmatched in detecting modifications like phosphorylation and

glycosylation, crucial for understanding protein function and disease mechanisms.[6]

• **Structural and Functional Proteomics:** Beyond identification, mass spectrometry offers insights into protein folding, interactions, and stability—key aspects in drug development and biomolecular research.[7]

• **Tissue and Cellular Proteomics:** Mass spectrometry has been instrumental in studying different cellular states and tissue-specific proteomes, fueling advances across the landscape of life science research including infectious diseases, neuroscience, cardiovascular diseases, and oncology.[8]

Despite decades of refinement, mass spectrometry-based proteomics remains largely confined to research labs due to the complexity of sample preparation, instrument operation, and data interpretation.[9] While genomics workflows have become increasingly automated and scalable, mass spectrometry-based proteomics still requires intricate workflows on expensive instrumentation with highly skilled operators, hindering its ability to make the same impact in clinical diagnostics, pharmaceutical development, and real-world applications.

Mass spectrometry also faces challenges in handling the vast dynamic range of abundance exhibited by proteins within biological systems. Without a PCR analog to amplify concentrations, low-abundance proteins, which often play critical roles in disease pathology and cellular function, are easily overshadowed by more abundant species.

Competitor technologies are advancing

Affinity-based approaches, such as aptamer and antibody arrays, have made significant strides in targeted protein detection. These methods offer high sensitivity and scalability, but their reliance on affinity reagent development and validation limits the rate of assay menu expansion. Current offerings for affinity-based approaches range from highly targeted with 10s of protein targets to the 11k SomaScan Assay measuring 10,000 unique human proteins.

Single-molecule detection techniques, such as nanopore and related direct sequencing technologies, promise to push proteomics into new territory, offering an alternative to mass spectrometry by directly sequencing proteins or measuring molecular interactions at an unprecedented scale. While still in their infancy, these approaches highlight the growing demand and interest for more accessible, high-throughput proteomic solutions.

At first glance, affinity-based approaches and mass spectrometry appear to be on equal ground; they yield comparable data quality and output in the context of discovery proteomics. Expansion of affinity-based assay menus (e.g., to 20,000 proteins plus proteoform specificity) will require large capital and time investments in reagent development and validation, but the next breakthroughs in mass spectrometry are already in sight. However, for mass spectrometry to reclaim its place at the forefront of biological discovery, fundamental shifts in its design and application are necessary.

• **Expanding Accessibility:** Current mass spectrometry platforms require expert users and extensive effort to operate. Future iterations must prioritize automation, streamlined workflows, reduced capital equipment costs, and user-friendly interfaces to broaden accessibility beyond core facilities.

• **Boosting Speed and Throughput:** Innovations in ion mobility and high-speed data acquisition are pushing mass spectrometry towards real-time proteomic analysis. There has been a noticeable, industry-wide shift toward faster QTOF architectures instead of the traditional, slower Orbitrap-based systems. By improving ion utilization and reducing redundancy, new approaches promise to increase efficiency without sacrificing depth.

• **Merging DIA and DDA Advantages:** Data-independent acquisition (DIA) offers unbiased broad coverage but generates complex datasets requiring sophisticated deconvolution. In contrast, data-dependent acquisition (DDA) provides high specificity but can miss low-abundance proteins. Integrating the strengths of both approaches into a single, high-performance workflow is critical.

• **AI and Computational Advancements:** The complexity of mass spectrometry data remains a major barrier to widespread adoption. AI-driven tools are now being developed to automate data analysis, improve confidence in protein identifications, and translate the raw data into biological insights.

Case study for the role of advanced ion utilization strategies

One major factor limiting the performance of conventional mass spectrometry approaches is poor ion utilization efficiency. Traditional fragmentation methods rely on quadrupole mass filters that discard the majority of available ions, dramatically decreasing sensitivity and proteome coverage. New methodologies are emerging to address this bottleneck by leveraging high-resolution ion mobility separation to pre-sort and isolate precursor ions before fragmentation. This approach enables significantly higher duty cycles, decreasing signal loss while maintaining spectral clarity.

One of the most promising advancements in this space integrates parallel accumulation strategies with mobility-aligned fragmentation, allowing for nearly complete ion utilization and enhanced fragmentation efficiency.[10] By ensuring that nearly all generated ions contribute to meaningful data, this technique not only improves sensitivity but also increases the depth of proteome coverage.

More importantly, it has the potential to resolve long-standing trade-offs between DIA and DDA, capturing the broad information depth of DIA while preserving the specificity and clarity of DDA spectra (*Figure 2*).

Furthermore, these approaches also introduce fundamental

changes to how data is generated and processed. The ability to fragment multiple precursor ions simultaneously at high speeds requires rethinking both instrument design and data processing workflows. This shift represents an opportunity to break free from legacy constraints and create a new paradigm in mass spectrometry-based proteomics—one that is faster, more efficient, and capable of truly unlocking the complexity of the proteome.

Realizing the promise of proteomics will require extending beyond simple protein expression studies focused on the relative concentration of proteins. Rather, the ultimate goal for maximizing biologically relevant insights is to unravel the true proteome where each canonical protein is a proteoform family on its own that encompasses PTMs, genetic variations, and alternative splicing isoforms for each expressed gene.[11]

As a result, the field of proteomics will fail to realize its full potential if it fails to fully integrate mass spectrometry into plasma proteomics to go beyond the limitations of affinity-based discovery workflows. Without mass spectrometry, proteomics risks falling behind in:

• **Biomarker Discovery:** Plasma is one of the richest sources for biomarkers, reflecting systemic and organ-specific disease states. Ignoring this resource would hinder disease prediction and diagnostics.

• **Non-Invasive Diagnostics:** Plasma sampling is minimally invasive, making it ideal for routine and longitudinal studies. In areas where affinity-based technologies are limited (e.g., PTM and proteoform specificity), increased reliance on more invasive and less practical methods will be required.

• **Personalized Medicine:** Protein biomarkers derived from plasma play a crucial role in early disease detection and treatment monitoring. Excluding mass spectrometry from this space would stall progress in tailoring therapies to individuals.

• **Translational Research:** Plasma proteomics is a direct bridge between laboratory research and clinical applications. Neglecting mass spectrometry in this area would slow the development of FDA-approved diagnostic tests and novel therapeutics.

• **Systemic Disease Monitoring:** Many conditions, such as autoimmune disorders and metabolic syndromes, require plasma analysis for a full understanding of disease progression.

The time is now for mass spectrometry to evolve, adapt, and lead the charge in unlocking the full potential of the proteome to improve human health. GEN

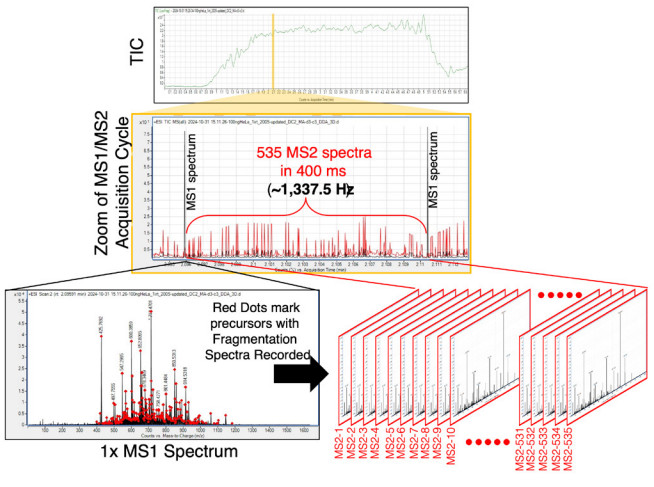


Figure 2. The PAMAF mode of operation uses high-resolution ion mobility to achieve >1,000 Hz MS2 spectral acquisition, delivering DIA-like data completeness with DDA-like high-quality fragmentation spectra for deeper and more accurate proteome coverage.

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DNA Methylation Sequencing

More Affordable Without Compromising Results

EpiCypher maps DNA methylation genome-wide with high sensitivity and low costs

By Aaron Alcala, PhD

DNA methylation is a highly studied epigenetic modification that is involved in regulating genome function and plays fundamental roles in development and disease.[1] It is linked to a broad range of conditions, including inflammation, neurological disorders, and cancer. Some patterns of methylation are shared across cancer types, while others can differ between subtypes—highlighting the value of studying DNA methylation to uncover novel biomarkers and gain insight into disease mechanisms.

Due to its chemical stability, DNA methylation can be analyzed across a range of sample types, including fresh, frozen, and formalin-fixed paraffin-embedded (FFPE) tissues. Further, noninvasive approaches such as liquid biopsy are being increasingly used to measure methylation patterns, enabling new methods for diagnosis and patient monitoring.

DNA methylation is also emerging as a promising therapeutic target, with several DNA methyltransferase inhibitors already approved for clinical use. Although several assays exist for mapping DNA methylation, better tools are needed to make these analyses more accessible and cost-effective for biomedical researchers.

Tradeoffs in DNA methylation mapping

Choosing the right method for DNA methylation profiling requires balancing several competing factors—cost, genome coverage, and resolution (*Table 1*). Each assay comes with its own tradeoffs, which not only impact data quality but also the feasibility of certain research applications.

Whole-genome bisulfite sequencing (WGBS) remains the gold standard for comprehensive DNA methylation analysis. It provides base-pair resolution of the methylome, making it ideal for in-depth studies of 5-methylcytosine (5mC) patterns across the genome. However, harsh bisulfite treatment damages DNA and skews GC coverage, introducing biases that can overestimate DNA methylation levels.

As a result, WGBS is expensive due to its requirement for high cell numbers and deep sequencing—often more than 800 million reads per sample. These demands can make WGBS impractical for large-scale studies or experiments using limited or precious samples.

Enzymatic approaches offer an alternative to bisulfite conversion, facilitating non-destructive mapping of DNA methylation. While enzymatic methods provide better genome coverage, reduced GC bias, and lower input requirements compared to WGBS, they still require high sequencing depths (>600 million reads per sample) and resource-intensive computational processing and bioinformatics expertise.[2]

Long-read sequencing technologies provide another approach that directly detects DNA methylation on native, unconverted DNA. Because long reads span kilobase-length fragments, they enable analysis of methylation in repetitive or structurally complex regions that are inaccessible to short-read platforms. Despite these advantages, long-read methods typically require the input of large amounts of intact, high-molecular-weight DNA and produce increased per-base error rates compared to short-read approaches.[3]

To reduce cost and complexity, researchers often turn to targeted approaches such as reduced representation bisulfite sequencing (RRBS), methylation arrays, or hybridization-based panels. These methods are more affordable but typically examine only a small subset of the methylome

Technology	Input	Coverage	Recommended Sequencing Depth	Turnaround Time	Base-Pair Conversion	Overall Cost
meCUT&RUN	10k - 500k cells	80% Methylated CpGs	>20 M reads	Days	Optional	$
RRBS	10 - 500 ng DNA	Limited (5-15% CpGs)	50-100 M reads	Weeks	Required	$$
Microarrays	250 ng DNA	Limited (~3% CpGs)	N/A	Days	Required	$$
Hybridization Panels	200 ng DNA	Limited (~15% CpGs)	>100 M reads	Weeks	Required	$$
WGBS	50 ng - 5 µg DNA	Whole genome	>800 M reads	Weeks	Required	$$$
EM-seq	100 pg - 200 ng DNA	Whole genome	>600 M reads	Days	Required	$$$
Long-read sequencing	>1 µg HMW DNA	Whole genome	>5 M long reads	Days	N/A	$$$

Table 1. Comparison of DNA methylation sequencing technologies.

(typically 3–15% of CpG sites) and tend to be biased toward CpG islands.[4,5] This limited scope may not provide the genome-wide coverage required for certain applications and can constrain the discovery of novel DNA methylation mechanisms.

Affinity-based techniques like methylated DNA immunoprecipitation sequencing (MeDIP-seq) are yet another strategy. This method uses antibodies to enrich for methylated DNA, reducing sequencing requirements compared to whole-genome DNA methylation profiling approaches. However, MeDIP-seq is technically challenging and has several limitations, including the requirement for high cell numbers and a preference towards hypermethylated and low-GC content regions.[4,6] Because this approach uses immunoprecipitation and often relies on poor quality 5-methylcytosine antibodies, MeDIP-seq suffers from poor reliability, resolution, and accuracy.

Together, these tradeoffs highlight the need for a more flexible methylation mapping approach that combines broad coverage and high sensitivity with lower sequencing requirements.

Cost-effective approach to mapping DNA methylation

To overcome the limitations of existing DNA methylation assays, researchers are turning to new methods that offer high-quality data at lower costs. One of these is EpiCypher's CUTANA™ meCUT&RUN, a novel assay that enables sensitive and cost-effective profiling of methylated DNA across the genome (*Figure 1*). This platform provides a flexible solution that balances coverage, resolution, and scalability.

To develop the meCUT&RUN workflow, the CUT&RUN protocol was modified to enrich for methylated regions. Instead of using antibodies, the assay uses a GST-tagged methylation-binding domain derived from human MeCP2 to selectively capture methylated DNA fragments. This strategy avoids harsh chemical treatments like bisulfite conversion, which can degrade DNA and introduce bias. As a result, meCUT&RUN is well-suited for limited or difficult-to-obtain samples, including clinical specimens and primary cells.

A key feature of this platform is its compatibility with different library preparation options, allowing researchers to tailor the assay to their specific needs (*Figure 1*). For those looking for the most efficient approach, enriched methylated DNA fragments can be directly sequenced to provide a comprehensive genome-wide map of DNA methylation.

Alternatively, researchers seeking base-pair resolution may add an enzymatic conversion step (NEBNext® EM-seq™ by New England Biolabs®) after methylated DNA enrichment, though this approach requires additional sample processing steps and computational analysis.

Compared to targeted approaches like RRBS, microarrays, and hybridization panels," meCUT&RUN delivers broader and more uniform methylome coverage with significantly lower sequencing requirements—achieving performance similar to EM-seq with

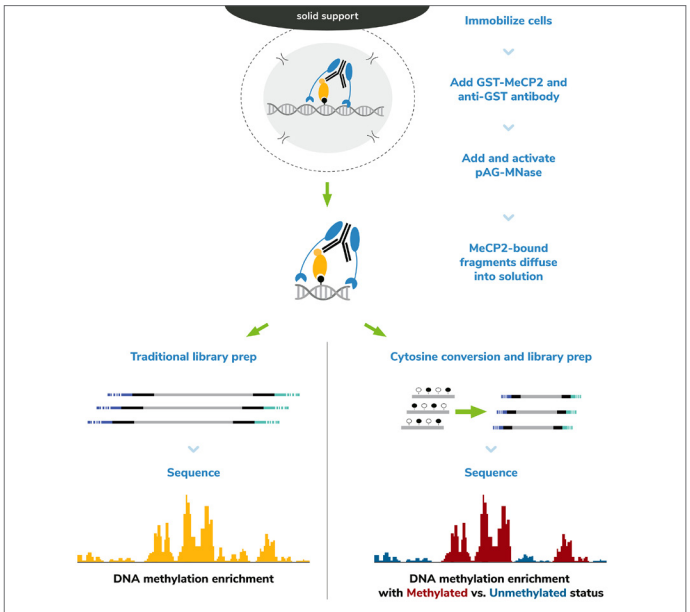

Figure 1. The modular meCUT&RUN workflow

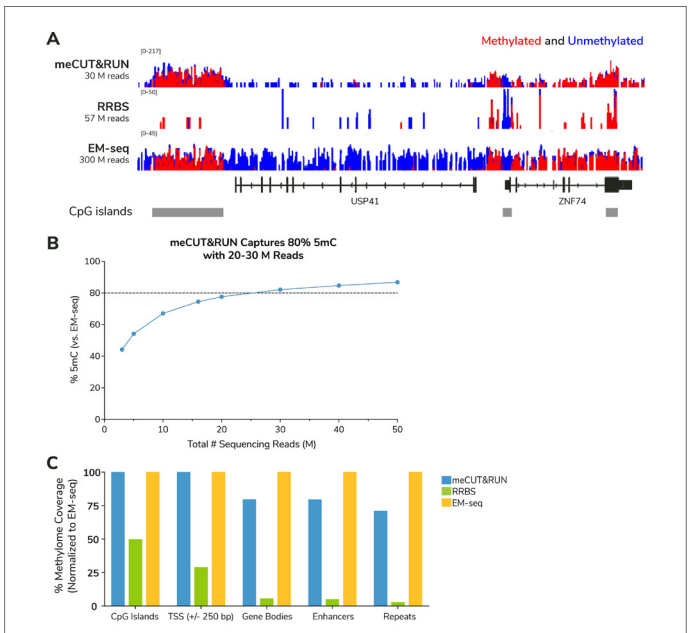

Figure 2. meCUT&RUN provides a high-quality snapshot of the human DNA methylome in K562 cells. (A) meCUT&RUN paired with EM-seq for base-pair resolution readout (first track). RRBS (ENCODE) and EM-seq data (EpiCypher) are shown for comparison. (B) meCUT&RUN recovers 80% of methylated CpGs (5mCs) compared to whole-genome EM-seq in K562 cells, with just 20-30 M unique reads. meCUT&RUN was paired with EM-seq for base-pair resolution 5mC analysis (meCUT&RUN-EM) and downsampled from 50 to 3 M uniquely aligned reads. The number of methylated CpG positions was calculated for each downsampled dataset and normalized to the number of 5mC positions detected by EM-seq. (C) Methylated CpGs detected by each method were assigned to respective genomic features; total counts were normalized to EM-seq data.

only 20–50 million reads (*Figure 2A*). Side-by-side comparisons show that meCUT&RUN identifies 80% of methylated CpGs (5mCs) captured by whole-genome EM-seq (*Figure 2B*). It also provides a more consistent detection of DNA methylation compared to RRBS across key genomic features, including enhancers, gene bodies, transcription start sites, and repetitive elements (*Figure 2C*).

Downsampling the number of cells used in the assay showed that the workflow performs reliably with as few as 10,000 cells per reaction (*Figure 3*), making it a more practical choice for larger studies or budget-conscious projects.

As interest in DNA methylation continues to grow across both basic and translational research, there is a pressing need for tools that offer flexibility, sensitivity, and affordability. meCUT&RUN provides an alternative to traditional assays by reducing sequencing burden while maintaining robust coverage and compatibility with low-input samples. By addressing common technical and cost-related challenges, this approach offers a practical solution for DNA methylation profiling that is accessible to a wide range of researchers. GEN

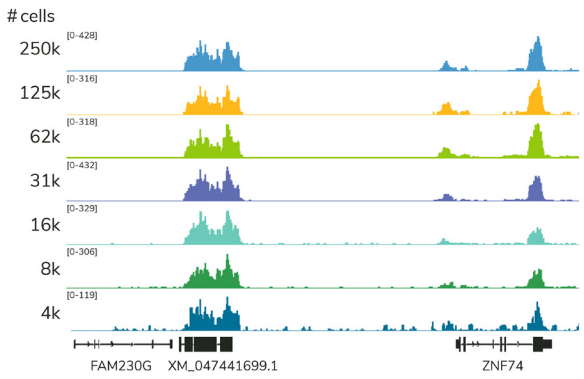

Figure 3. Robust performance of meCUT&RUN at lower cell inputs. Methylation profiles remain consistent down to 8,000 K562 cells, comparable to those generated with 500,000 cells.

Aaron Alcala, PhD, is a scientific grant writer for EpiCypher.

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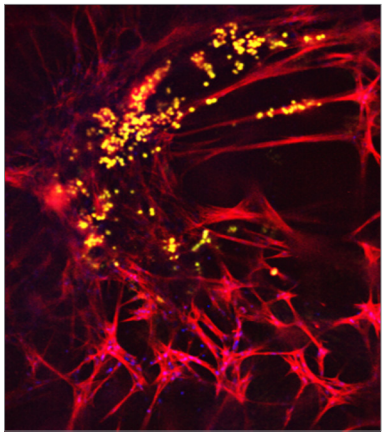

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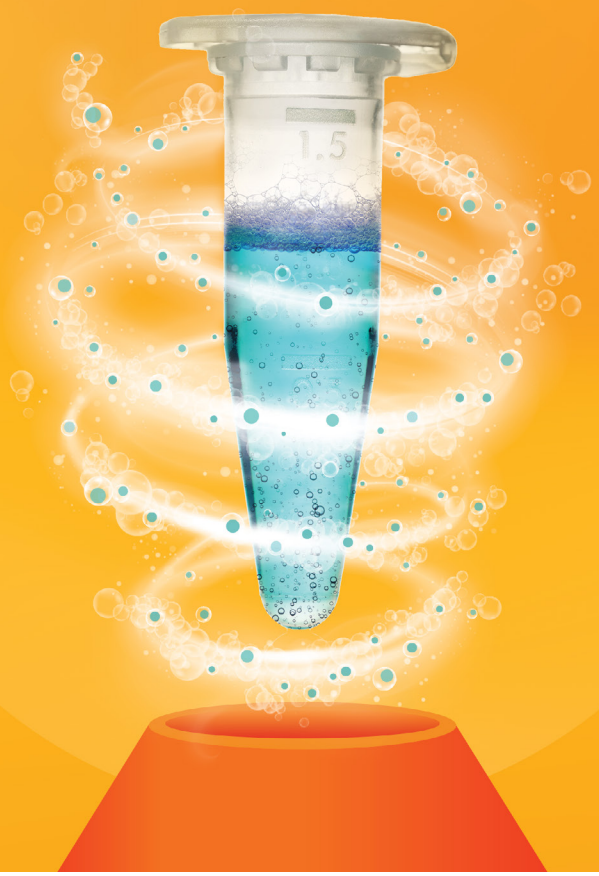

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