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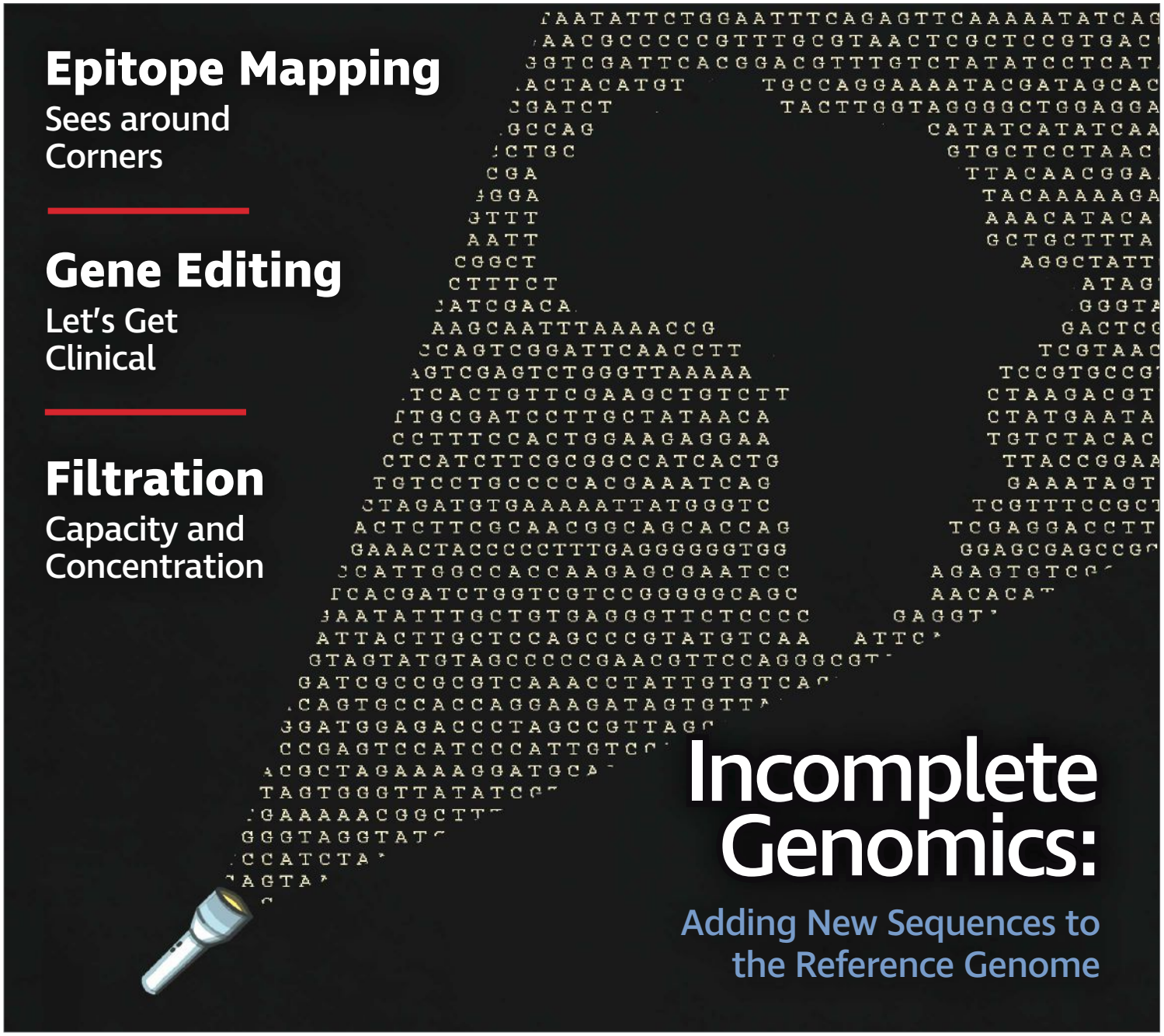

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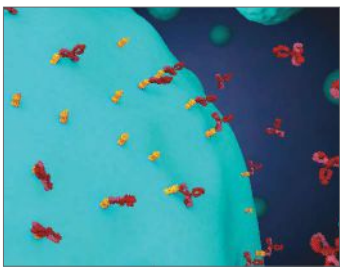
FEBRUARY 2019 | Volume 39 | Number 2

CONTENTS

OMICS FEATURE

DRUG DISCOVERY



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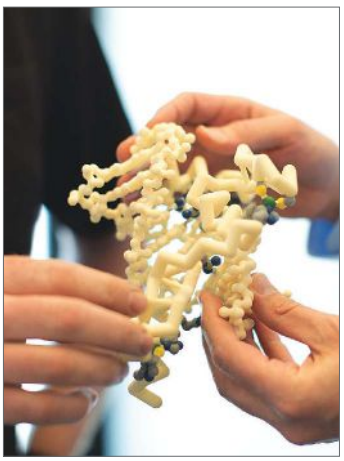

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Gut Microbiome–Pomegranate Partnership Reduces Colitis

Pomegranates are believed to have potentially manifold benefits to human health.

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NEWS

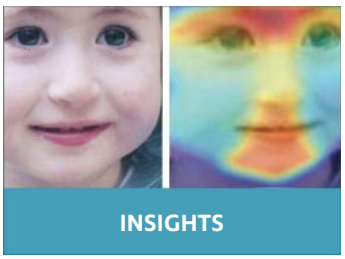
Inhaled mRNA Therapy Could Be Breath of Fresh Air for Lung Disorders

Delivery mode for mRNA inspires pulmonary therapeutics.

NEWS

NSAID-Altered Microbiome Exacerbates *C. diff* Infections

The most common hospital-acquired infection has become a global public health issue.



INSIGHTS

AI Gets in the Face of Rare Genetic Diseases

A face recognition program takes the guesswork out of diagnosing genetic disorders.

CONTENTS

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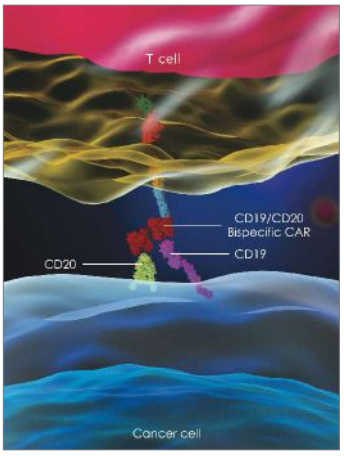
BIOPROCESSING

TRANSLATIONAL MEDICINE



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From the Editor in Chief

John Sterling

In 2007, James Watson, PhD, co-discover of the structure of DNA, startled the world in an interview with *The Sunday Times* of London when he said he was "inherently gloomy about the prospect of Africa ... all our social policies are based on the fact that their intelligence is the same as ours, whereas all the testing says not really." Pouring more oil onto the social fire he lit, Watson added that while everyone wants human beings to be equal, "people who have to deal with black employees find this not true."

In a documentary that aired on PBS last month, Watson would not retract these statements even when given the chance. Long associated with the Cold Spring Harbor Laboratory, Watson was relieved of all his administrative duties and had his status as chancellor eliminated 12 years ago. After the PBS documentary, he lost his remaining titles at the Lab.

What is surprising about all this is how a geneticist of Watson's status and intelligence could utter such hogwash when there is absolutely no, nada, zippo, zilch scientific evidence to support the intellectual superiority of one group or race (which is not a true biological term) over another. Watson's remarks, however, represent the latest examples in a long history of Western scientific racism.

For example, several French scientists and pseudoscientists in the 18th and 19th centuries were among the first to try to link skin color to human behavior and abilities. Even Charles Darwin believed in racial hierarchies, demonstrating that on some topics, he held views that were, lamentably, unexceptional for his time—a point recognized by many contemporary writers, including Steven Rose, PhD, currently emeritus professor of biology and neurobiology at Open University. In an opinion piece contributed to *EMBO Reports* in 2009, Rose wrote that Darwin was "convinced that evolution was progressive, and that the white races—especially the Europeans—were evolutionary more advanced than the black races."

Also of their time and place were exponents of Social Darwinism such as William Graham Summer, Herbert Spencer, Karl Pearson, and Benjamin Kidd, as well as Francis Galton, who developed the forerunner to the Stanford-Binet IQ test. They were committed to showing "scientifically" that Europeans and White Americans were superior to Africans, Asians, Latin Americans, and Native Americans. More recently, the late psychologist and eugenicist Arthur Jensen resorted to pseudoscience to try to make a case for black intellectual inferiority.

Over the last 200 years, solid scientific and cultural research all around the globe has clearly demonstrated that no claim can be sustained that biological races exist or that specific human populations are genetically or intellectually superior to others. Nevertheless, so-called race science is resurgent in some quarters, and white nationalist sentiments are on the rise in Europe and the United States.

These erroneous and dangerous ideas are being opposed, as they must, by people of wisdom and good will. Ignorant statements from a once-esteemed scientist like Dr. Watson do nothing but help set back the cause.

John Sterling
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Catalent Invests $200 Million in Expansion

Catalent says the $200 million it will spend over three years to expand drug substance manufacturing capacity and drug product fill/finish capacity in Bloomington, IN, and Madison, WI, reflects a commitment to expand its fast-growing biologics business further.

Work has begun at both sites, with completion expected in mid-2021. The provider of advanced delivery technologies and development solutions for drugs, biologics, and consumer health products cited projected growth among existing and future customers in announcing the expansion this week.

Catalent, a provider of delivery technologies and development solutions for drugs, biologics, and consumer health products, is expanding its facilities in Madison, WI, and Bloomington, IN. The expansion effort is expected to span three years and leverage a $200 million investment.

"We're still seeing strong growth in demand for development and production of proteins and monoclonal antibodies," Michael A. Riley, Catalent VP, general manager, drug substance & bioanalytical, told *GEN*, in an interview during the J.P. Morgan 37th Healthcare conference, held in San Francisco. "We're really trying to invest to get out in front of that demand. That's demand from current customers as well as the market in general."

Biologics accounted for 26% of the $2.463 billion in total revenue Catalent reported for its 2018 fiscal year, which ended September 30—nearly double the 14% of FY 2017.

Riley said the company wants to grow the percentage of its business that is biologics-driven, but won't say how much higher it is looking to grow that business.

In October 2017, Catalent signaled its intent to expand in biologics manufacturing by completing its $950 million acquisition of Cook Pharmica, gaining control of the Bloomington facility as a result.

"This expansion investment and other investments like the Cook acquisition is a signal that Catalent is putting significant weight on biologics as part of a growth strategy for the company," Riley said.

In Bloomington, Catalent intends to hire up to 200 people by the end of 2024, expanding a facility that now has a workforce of 900 people. The company's expansion plans for the site include:

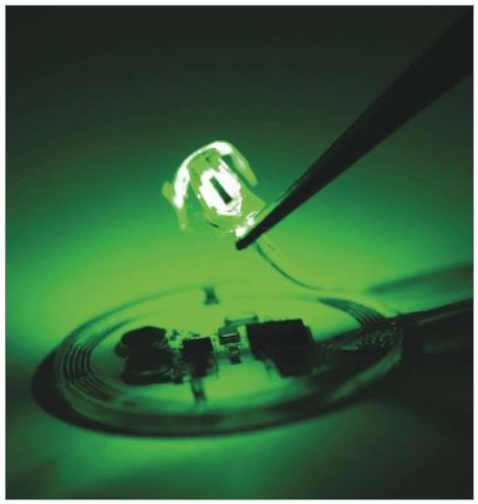
- Installing a high-speed flexible vial line, utilizing both ready-to-use (RTU) components and bulk filling, at a filling speed of 300 units per minute.
- Installing a high-speed flexible syringe/cartridge line with a filling speed of over 300 units per minute, as well as a fully automated vial inspection machine.
- Expanding fill/finish capacity by 79,000 square feet, with both GMP and non-GMP capabilities—adding space to the 875,000-square-foot biologics development and manufacturing facility.

The Bloomington site is designed to offer Catalent clients its expertise in sterile formulation and extensive biomanufacturing and drug product fill/finish capacity across liquid and lyophilized vials, prefilled syringes, and cartridges. In December 2018, Catalent said it would invest nearly $14 million to build a 15,000-square-foot biologics packaging facility intended to expand its capabilities and capacity. ■

Optogenetics Greenlights Bladder Control Therapy

Effective treatments for incontinence are urgently needed. A multi-institutional team of researchers has developed a wireless device that can measure bladder function in freely moving rats over days to weeks. It can then use that real-time information to identify pathological voiding behavior and deliver a corrective signal in real time, in a closed-loop fashion.

Once implanted, the device provides "set it and forget it" neuromodulation that lessens the urge to urinate, and does so only when necessary and without any outside intervention. The approach, which uses optogenetics, soft electronics, data analytics, and wireless powering/communication technologies to address overactive bladder, was described in a paper



An implantable optogenetics device can activate nerve cells in the bladder and control problems such as incontinence and overactive bladder. The device, which uses light signals from tiny LEDs, was demonstrated in a rat model by scientists based at Washington University in St. Louis.

("A wireless closed-loop system for optogenetic peripheral neuromodulation") published January 2 in *Nature*.

"When the bladder is emptying too often, the external device sends a signal that activates micro-LEDs on the bladder band device, and the lights then shine on sensory neurons in the bladder. This reduces the activity of the sensory neurons and restores normal bladder function," Robert W. Gereau IV, PhD, professor at Washington University in St. Louis and a senior author, tells *GEN*.

The device is an implanted strain gauge (a soft, stretchy belt-like device) that wraps around the bladder. It is engineered so that the electrical resistance increases as it stretches (when the bladder is filling) and decreases as it relaxes (when the bladder empties).

When the device is activated, the LED turns on. The light from the LED activates an opsin (a light-sensitive protein) called archaerhodopsin, which is expressed in the sensory neurons, innervating the bladder neurons using a viral gene therapy vector. When archaerhodopsin is activated, it changes the properties of the nerve membranes causing inhibition of neuronal firing. Thus, the LED activation results in specific inhibition of the neurons that send signals from the bladder to the brain.

Rogers and Gereau envision that a reasonable prototype could be available within a couple of years, but the researchers note that safety concerns must be addressed. ■

Value-Based Pricing's Bid to Commodify Cell and Gene Therapies

A *cynic*, according to one definition, is someone who knows the price of everything and the value of nothing. If that is so, then a cynic would be ill-equipped to assess the value of value-based pricing, particularly as it applies to life-saving cell and gene therapies. These therapies, which may cure devastating diseases after just one application, could be considered priceless. Yet a one-time gene therapy, for example, does have a price, one that is so high that a one-time payment would be out of the question.

Not a problem, you might say. A ready solution exists: the installment plan. But that solution leaves another problem unresolved. While individual patients would pay any price, if they had the means or a payer capable of paying on their behalf, a healthcare system, or even the larger society, might suffer sticker shock.

It is this problem for which value-based pricing solutions have been proposed. A well-established strategy for pricing commodities, the value-based approach focuses on the value to the buyer, not the actual costs of production (plus a margin). It should be emphasized that the buyer needn't be construed as the individual patient. The buyer could, in a sense, be everyone who derives a secondary benefit from a life saved or from a life lived free of debilities that would impose suffering or call for resource-consuming measures. The buyer could also be anyone who would benefit if workable reimbursement plans succeeded in encouraging developers to continue seeking new cures.

So, if everyone has skin in the game, why shouldn't everyone pitch in? Or, put in different terms, why shouldn't everyone share some of the risk that a one-time treatment will fail to deliver the expected value? This last question gets to value-based pricing's latest wrinkle: the pay-for-performance pitch. It has the potential to put the producer of a cell or gene therapy on the hook, along with the rest of us.

A pay-for-performance arrangement was recently proposed by **bluebird bio**, a provider of lentiviral-based gene therapies, T-cell immunotherapy expertise, and gene editing capabilities. Last month, at the 37th Annual J.P. Morgan Healthcare Conference, a bluebird bio presentation suggested that reimbursement systems, no less than patients' genomes, could benefit from recoding.

The company declared that it is "willing to share the risk of uncertainty to prove the lifelong value of its therapies," including its LentiGlobin gene therapy, which is currently in Phase III testing and promises to benefit patients with transfusion-dependent β-thalassemia. In particular, bluebird bio floated a reimbursement plan that not only spreads payments over a five-year period, but also calls for payments to cease should treatment milestones be missed. By making payments contingent on patient quality-of-life and life-extension measures, bluebird bio aspires to be a "catalyst for change" and "establish a sustainable model for pricing and reimbursement of gene therapies." ■

Brendon Thorne/Stringer/Getty Images

StickyEnds

Dog Personalities Are Nature Not Nurture

If you're a fan of a specific dog breed, you can thank its DNA, suggest researchers from the Universities of Arizona, Washington, and Pennsylvania, and Princeton University. They looked at heritable traits across an array of breeds. Writing in a *bioRxiv* preprint, the researchers noted that they looked at "behavioral data from more than 17,000 dogs from 101 breeds with breed-averaged genotypic data ($N = 5697$ dogs) from over 100,000 loci in the dog genome."

What the scientists found were 131 single nucleotide polymorphisms in the canine genome that appear to influence the development of 14 dog personality traits—such as trainability, stranger-directed aggression, chasing, and attachment and attention.

Interestingly, the findings suggest that canine behavior may stem from gene homologs linked to behavior in many other species, even humans. Since this is just an initial foray into understanding genetic, behavioral attributes in dogs, the researchers didn't link any breeds to specific behavioral propensities—but the researchers feel they are barking up the right tree and looking to fetch new genetic data soon.

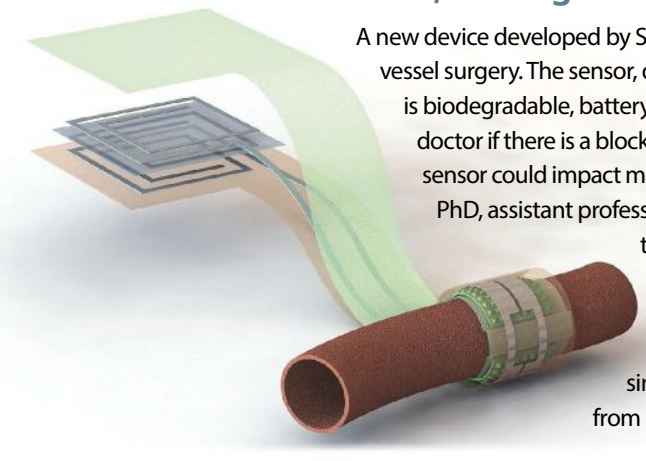
Race Plays Important Role in Breast Cancer Prevention

African-American women at high risk of breast cancer are less likely than white women to pursue potentially life-saving preventive care, and racial disparities in healthcare and elsewhere are to blame, new research suggests. "African-American women faced additional burdens at every step along the risk-management journey," researchers from Ohio State University wrote in a newly published study in *Ethnicity & Health*.

The study included in-depth interviews with 50 women (30 white, 20 black) deemed at high risk of breast cancer based on family history and other factors. The team found that high-risk black women were less likely than white women to have genetic testing, take medications to protect them against cancer, and to have (or consider having) their breasts or ovaries removed as a preventive measure, disparities that have been seen in previous studies. For example, 67% of white study participants said they or a relevant family member had undergone genetic testing, whereas just 20% of black women reported a history of genetic testing.

These trends could help inform care provided to women in the future, prompting providers to acknowledge and look for ways to clear obstacles to lowering breast cancer risk for patients.

Wireless, Biodegradable Blood Flow Sensor



A new device developed by Stanford University researchers could make it easier for doctors to monitor the success of blood vessel surgery. The sensor, detailed in a *Nature Biomedical Engineering* paper, monitors the flow of blood through an artery. It is biodegradable, battery-free, and wireless, so it is compact and doesn't need to be removed, and it can warn a patient's doctor if there is a blockage. "Measurement of blood flow is critical in many medical specialties, so a wireless biodegradable sensor could impact multiple fields including vascular, transplant, reconstructive, and cardiac surgery," said Paige Fox, MD, PhD, assistant professor of surgery and co-senior author of the paper. "As we attempt to care for patients throughout the Bay Area, Central Valley, California, and beyond, this is a technology that will allow us to extend our care without requiring face-to-face visits or tests."

Monitoring the success of surgery on blood vessels is challenging, as the first sign of trouble often comes too late. By that time, the patient often needs additional surgery that carries risks similar to the original procedure. This new sensor could let doctors keep tabs on a healing vessel from afar, creating opportunities for earlier interventions.

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The Art of Biotech Customization

By Gail Dutton

Deep discussions at Roche CustomBiotech lead to innovation and customer-focused solutions

GMP—good manufacturing practice—is such a common term in bioprocessing that its precise meaning is becoming clouded. Such ambiguity can create problems across organizations.

Just because multiple parties follow good manufacturing (or good lab) practices doesn't mean they all follow the same practices. Minute differences can determine whether manufacturers meet existing regulatory requirements or are prepared for likely future requirements. Addressing differences early helps minimize misunderstandings among suppliers and customers regarding their efforts, timelines, and costs on the way to commercialization.

Roche CustomBiotech has seen problems evolve from such discrepancies often enough to know that it and its customers must have a mutual understanding of the details that go into the making of high-quality life sciences products. Achieving transparency with its customers, therefore, is one of Roche CustomBiotech's distinguishing elements.

Christian Huber

"The biggest challenges for the diagnostics and bioprocessing industry is proving that everything goes well from the start and all the way through clinical trials and commercialization," elaborates Christian Huber, international business leader for Roche CustomBiotech. And the way to meet those challenges is to focus on the details to ensure they each are right. That includes having the ISO 13485 certificate, proving quality by adhering to GMP where applicable, documenting each detail, and demonstrating scalability. "We're at the top end of what we do and how we share transparency. Having those conversations about GMP, scalability, and a host of other details is part of what sets us apart."

Driving a paradigm shift

Roche traces its beginnings to 1896, when Fritz Hoffmann-La Roche launched one of the world's first industrial-scale pharmaceutical companies. As the company grew, it added offices throughout the world and expanded the business to include pharmaceuticals, vitamins and fine chemicals, diagnostics, and flavors and fragrances. Nearly 100 hundred years later, the company returned to its

Roche CustomBiotech's headquarters in Penzberg, Germany, merges diagnostics and pharmaceutical knowhow in one location. Constituting one of the largest biotechnology centers in Europe, the Penzberg facility is both a multidisciplinary incubator of novel technologies for production and automation processes, and a state-of-the-art manufacturer of high-quality raw materials and biotechnology products.

VITAL SIGNS

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Number of Employees
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Focus
Roche CustomBiotech develops industrial-scale solutions for the biopharma and diagnostics industry, ranging from raw ingredients through systems solutions for bioprocessing.

original focus: pharmaceuticals and diagnostics. At about that time, in the 1980s, Roche CustomBiotech was formed.

"The purpose, originally, was to sell select products and solutions into the diagnostics and biopharmaceutical markets. We saw a chance to increase sales and use excess manufacturing capacity. It became clear, however, that we could do more than sell off the shelf. Then custom projects came into the picture," Huber says. Product variations morphed into opportunities for co-development that leveraged Roche's R&D and operations skills. Gradually, those opportunities led to the adaptation of in vitro diagnostics instruments and assays for metabolite testing and bioprocess monitoring, as well as technology platforms for the diagnostic side of the business.

Biotherapeutics and diagnostics each have strong growth potential, he says, citing the steady expansion of molecular and sequencing options to shed light on disease, and physicians' growing reliance on companion diagnostics, as well as the use of big data analytics to match the right drugs to the right patients for clinical trials for increasingly personalized medicine.

Roche CustomBiotech's stated mission is, according to Huber, "to enable the healthcare industry to develop and produce state-of-the-art solutions for diagnosis and treatment. We do that by providing reliable, tailor-made, high-quality products and technologies." To that end, the company provides raw materials, instrumentation, products, and services for drug development, production, cell therapy, and in vitro diagnostics for its clients, customized to clients' specifications.

mRNA interest grows

CustomBiotech is particularly interested in providing enzymes for mRNA therapeutics—both as cancer vaccines and drugs. This relatively new therapeutic option offers an attractive alternative to traditional therapies and, although only a relatively few clinical trials are underway globally, seems to suggest increased efficacy.

Roche CustomBiotech's Cedex Analyzers monitor cell culture conditions inside bioreactors. Members of the Cedex family include the Cedex Bio Analyzer (*front, center*), the Cedex Bio HT Analyzer (*back, left*), and the Cedex HiRes Analyzer (*right*). In-process measurements can reveal changes in nutrients, metabolites, cell morphology, and cell growth.

As to the potential value for therapeutic mRNA, Huber points to **Moderna Therapeutics'** IPO on December 6, which raised $604 million in what is widely touted as the largest-ever U.S. biotech IPO.

"We want to be a leading supplier for therapeutic mRNA manufacturers," Huber says. CustomBiotech's mRNA enzyme portfolio has grown steadily during the past 18 months, including the T7 RNA polymerase as the main enzyme. A new RNAse inhibitor will be launched next year, and "we're continuing to develop new enzymes to complement our portfolio. Therapeutic mRNA regulations are evolving and may require higher quality levels in the future," he points out.

Product variations morphed into co-development opportunities that leveraged Roche's R&D and operations skills.

The bioprocess monitoring business also is seeing increased investment from the company in the form of a range of in-process control testing. "We also will expand and build on our bioprocess monitoring with our Cedex line of metabolite and cell analyzers," Huber says. Currently, this product line includes the Cedex Bio HT Analyzer, Cedex Bio Analyzer, and the Cedex HiRes Analyzer. These may be integrated into the cell fermentation process.

On the diagnostics side, CustomBiotech is also expanding its range of molecular diagnostics with novel engineered polymerases, such as KAPA 2G and KAPA 3G. The benefit, Huber explains, is that "these second- and third-generation enzymes allow for a faster and more inhibitor resistant amplification reaction, generating fast and highly valid test results from crude prepared samples."

Customization and scalability

Roche CustomBiotech is known for its customization and for the scalability of its solutions. "We've developed more than 150 custom solutions including many co-developments in the past 10 years," Huber points out. "We have shared histories with a number of companies that have become market leaders, and we have grown with them."

Such successes for Roche and its customers imply a plenitude of discussions with organizations at the forefront of their fields. Their purpose is to understand innovators' goals, more immediate needs, and, of course, how Roche CustomBiotech can help. Those continual communications, leveraging Roche CustomBiotech's expertise, in-depth market research, and attendance at industry events all contribute to this ability, showing that thoughtful conversations really can catalyze innovation. GEN

Tissue Engineering Market in the U.S.*

By Yu Seon Kim, Mollie M. Smoak, Anthony J. Melchiorri, PhD, and Antonios G. Mikos, PhD

In the $9 billion/year market for tissue engineering products, 21 of 49 companies generate the revenue

All the authors represent Rice University: Y.S.K. and M.M.S. are graduate students; A.J.M. is associate director, Biomaterials Lab; and A.G.M. is Louis Calder Professor of Bioengineering and professor of chemical and biomolecular engineering.

Since the term tissue engineering was coined in 1993, the fields of tissue engineering, regenerative medicine, and cell therapy have greatly matured from benchtop ideas to commercially available products that are widely used in the clinic. We broadly define "tissue engineering" as the culmination of engineering and biology to advance the restoration or improvement of tissue function.

Artificial organs, biomaterials, and cell therapies that leverage autologous or donor cells have been utilized for regenerative purposes. Currently, the terms "tissue engineering" and "regenerative medicine" bring up 1.2 and 0.6 million results on Google Scholar, respectively. This indicates how much the field has grown in only 25 years.

This report seeks to provide an update of the tissue engineering industry from 2011 to 2018. Public tissue engineering companies with a presence in the United States were the focus of this report due to the publicly accessible financial data that they provide. The report identifies 49 companies. (A partial list, which shows 25 companies, appears in the *Table*.) Twenty-one of the companies were in the commercial phase of development and had products on the market. These 21 companies made an estimated $9 billion in sales of tissue engineering–related products in 2017. Based on previous reports and market trends, the field of tissue engineering is forecasted to continue to build revenue for the years to come. GEN

*Adapted and abridged from "An Overview of the Tissue Engineering Market in the United States from 2011 to 2018," *Tissue Engineering, Part A*, September 4, 2018, published by Mary Ann Liebert, Inc.

Table. Public Companies in the Tissue Engineering and Regenerative Medicine Sector as of March 2018*

Company name	Location	Sector	Stage	Website
1. Acer Therapeutics	Newton, MA	Stem cells	Clinical trials	www.acertx.com
2. Allergan	Madison, NJ	Biomaterials	Commercial	www.allergan.com
3. Alliqua Biomedical	Yardley, PA	Biomaterials	Commercial	www.alliqua.com
4. American CryoStem	Red Bank, NJ	Stem cells	Service	www.americancryostem.com
5. Anika Therapeutics	Bedford, MA	Biomaterials	Commercial	www.anikatherapeutics.com
6. Asterias Biotherapeutics	Fremont, CA	Stem cells	Clinical trials	www.asteriasbiotherapeutics.com
7. Athersys	Cleveland, OH	Stem cells	Clinical trials	www.athersys.com
8. Avita Medical	Valencia, CA	Cells and biomaterials	Commercial	www.avitamedical.com/us
9. Axogen	Alachua, FL	Biomaterials	Commercial	ir.axogeninc.com
10. Biocardia Lifesciences	San Carlos, CA	Cells and biomaterials	Commercial	www.biocardia.com
11. Biorestorative Therapies	Melville, NY	Cells and biomaterials	Preclinical	www.biorestorative.com
12. BioTime	Alameda, CA	Stem cells	Clinical trials	www.biotimeinc.com
13. BrainStorm Cell Therapeutics	New York, NY	Stem cells	Clinical trials	www.brainstorm-cell.com
14. Caladrius Biosciences	Basking Ridge, NJ	Stem cells	Clinical trials	www.caladrius.com
15. Capricor	Beverly Hills, CA	Cells and biomaterials	Clinical trials	www.capricor.com
16. Celgene[1]	Summit, NJ	Stem cells	Service	www.celgene.com
17. Celyad	New York, NY	Stem cells	Clinical trials	www.celyad.com
18. Cesca Therapeutics	Rancho Cardova, CA	Stem cells	Service	www.cescatherapeutics.com
19. Cord Blood America	Las Vegas, NV	Stem cells	Service	www.cordblood-america.com
20. CryoCell	Oldsmar, FL	Stem cells	Service	www.cryo-cell.com
21. Cryolife	Atlanta, GA	Biomaterials	Commercial	www.cryolife.com
22. Cytori Therapeutics	San Diego, CA	Stem cells	Commercial	www.cytoritx.com
23. Exactech	Gainesville, FL	Biomaterials	Commercial	www.exac.com
24. Fate Therapeutics	San Diego, CA	Stem cells	Clinical trials	www.fatetherapeutics.com
25. Geron	Menlo Park, CA	Stem cells	Clinical trials	www.geron.com

* To view a PDF document of the full table (all 49 companies), please visit **www.GENengnews.com**.

1. At press time, Bristol-Myers Squibb was in the process of acquiring Celgene.

To view the original report, please visit
https://www.liebertpub.com/doi/10.1089/ten.tea.2018.0138

10 Takeover Targets to Watch in 2019

By Alex Philippidis

Blockbuster deals suggest a healthy year for biopharma M&A

After weeks of speculation that biopahrama dealmaking would cool off, 2019 got off to a flying start as far as mergers-and-acquisitions (M&A) is concerned, thanks to two blockbuster transactions: **Bristol-Myers Squibb's** planned $74 billion acquisition of **Celgene**, followed four days later by **Eli Lilly's** planned $8 billion purchase of **Loxo Oncology**—a deal that quickly became the buzz of the J.P. Morgan 37th Healthcare Conference, held earlier this month in San Francisco.

During a "breakout" Q-and-A with analysts following a town-meeting-style address at the conference, Lilly chairman and CEO David A. Ricks cautioned that, for his company at least, size isn't necessarily what matters most when it comes to acquisitions: "I think scale probably destroys more value than it creates, particularly in R&D functions," Ricks said, adding: "What matters is differentiation of assets." Lilly has "plenty of capacity" to do acquisition deals every quarter, Lilly SVP and CFO Joshua L. Smiley added.

However, Ricks added that he foresaw an increase in M&A activity in 2019. He noted the mixed results shown by smaller biopharmas when it comes to successfully commercializing their own drugs once they reach the market— as well as the decline in market capitalization of biopharmas, which slumped along with most other public companies late last year.

Which biopharmas will be the next ones to be acquired? *GEN* has sought to answer that question in recent years through lists of takeover targets that have generated the most M&A buzz based on notes to investors and comments in news outlets.

GEN has had some success predicting companies on the cusp of being bought out. *GEN's* February 26, 2018, list included **AveXis** just two months before it found a buyer in **Novartis**, which completed its $8.7 billion acquisition of the gene therapy developer on May 15. Another company on that list, **Tesaro**, revealed December 3 it is being snapped up by **GlaxoSmithKline** for $5.1 billion. Only one of the 10 companies on our 2017 list found a would-be buyer (**Juno Therapeutics**, purchased by Celgene).

EvaluatePharma has tracked $136.5 billion in 173 biopharma M&A deals during 2018, compared with $79 billion in 183 deals in 2017. Last month, Informa Pharma Intelligence told *GEN* it counted about $265 billion in 2018 M&A, up 26% from $210 billion in 2017—including nontraditional "disruptive" healthcare deals such as the pending $69 billion CVS-Aetna merger. GEN

See more lists online
GENengnews.com/lists

1 Alexion Pharmaceuticals

Boston, MA
www.alexion.com

Alexion Pharmaceuticals made a big splash late last year with a planned **up-to-$1.2B acquisition of Syntimmune and $637M-plus collaboration with Dicerna Pharmaceuticals** to discover and develop RNA therapies for complement-mediated diseases. Ronny Gal at Sanford Bernstein told *MarketWatch* the company would appear a logical takeover target for Amgen, while Bret Jensen in *Seeking Alpha* called out BioMarin Pharmaceutical (see next page) as a potential buyer. On December 10, Zacks Investment Research named Roche, Pfizer, or Novartis as having interest in buying Alexion.

2 Alnylam Pharmaceuticals

Cambridge, MA
www.alnylam.com

Alnylam made biopharma history in August 2018, **winning the FDA's first-ever approval for an RNAi treatment with Onpattro™ (patisiran)**. Kinjel Shah of Zacks Investment Research and market watcher Barry Cohen are pinpointing Sanofi as a likely buyer since its Sanofi Genzyme subsidiary bought $700M in Alnylam stock when the companies began partnering in 2014. Shah cited Alnylam's two late-stage pipeline candidates—inclisiran (partnered with The Medicines Company) for hypercholesterolemia, and givosiran for acute hepatic porphyria.

3 Amarin

Dublin, Ireland
www.amarincorp.com

During Q1, Amarin plans to submit a supplemental NDA for fish-derived Vascepa® (icosapent ethyl), which **aced the Phase III REDUCE-IT™ trial by generating a 25% relative risk reduction** in first occurrence of major adverse cardiovascular events. *StreetInsider* reported January 10 Pfizer was preparing to bid for Amarin. On October 16 in InvestorPlace, Luke Lango of San Diego-based L&F Capital Management called Amarin "an exceptionally attractive M&A target" for Amgen, Regeneron, and Sanofi.

4 BioMarin Pharmaceutical

San Rafael, CA
www.biomarin.com

Chairman and CEO Jean-Jacques Bienaimé has said BioMarin Pharmaceutical **will submit a BLA in 2H 2019 for valoctocogene roxaparvovec**, a gene therapy Factor VIII for hemophilia A, and pursue European approval for Palynziq® (pegvaliase) for patients 16+ with phenylketonuria (PKU). "It is a comparatively low-risk acquisition for a big pharma firm looking to add to its drug offerings and fend off the decline in revenue associated with patent expiration and competition," analyst Jeff Reeves wrote January 10 in *MarketWatch*.

5 Clovis Oncology

Boulder, CO
www.clovisoncology.com

AstraZeneca's planned **$5.1B acquisition of Tesaro led to buzz about acquisitions of other PARP inhibitor developers**—including Clovis Oncology. Rubraca® (rucaparib) product revenue jumped 69% during Q1–Q3 2018 year-over-year to $65M, yet lags behind competing PARP inhibitors. "[Clovis] will become the only standalone PARP, which we believe makes it the most likely takeout target in 2019," Gabelli analyst Jing He told investors. He identified three "most interested" potential buyers: Bristol-Myers Squibb, Sanofi, and Roche.

6 Gilead Sciences

Foster City, CA
www.gilead.com

Bristol-Myers Squibb's planned **$74B acquisition of Celgene** sparked speculation about more big biotech takeovers. Oppenheimer analyst Hartaj Singh has cited Gilead's potential to attract a buyer, and improve results under incoming chairman and CEO Daniel O'Day. Gilead is counting on positive Phase III data for filgotinib, being co-developed with Galapagos, in Crohn's disease and ulcerative colitis. Michael Yee of RBC Capital Markets added Gilead to a short list of buyout candidates that included Alexion, Biogen, and Vertex Pharmaceuticals.

7 Global Blood Therapeutics

South San Francisco, CA
www.gbt.com

Global Blood Therapeutics (GBT) **is basking in the investor glow of positive Phase III results** for oral, once-daily sickle cell disease candidate voxelotor on December 3, which **sent shares soaring 48% to $46.62** (shares closed at $48.87 on January 21). An accelerated NDA is planned in 2H 2019. Analysts Jared Holz of Jefferies and Michael Yee of RBC included GBT among six potential buyout targets January 15 in *MarketWatch*. Wedbush analyst Liana Moussatos projects $1B+ in voxelotor sales by 2023.

8 Incyte

Wilmington, DE
www.incyte.com

Incyte's JAK inhibitor **Jakafi® (ruxolitinib) exceeded $1B in net product revenue** during Q3 2018. Jakafi has two rare blood cancer indications in polycythemia vera and myelofibrosis—and could add steroid-refractory acute graft-versus-host disease, depending on FDA Priority Review; Incyte's PDUFA date is February 24. Also rekindling buyout interest is the prospect of additional indications for Eli Lilly-partnered arthritis treatment baricitinib. Incyte was among 10 biopharma takeover targets cited by Bloomberg in a survey of trading desks, analysts, and fund managers.

9 Neurocrine Biosciences

San Diego, CA
www.neurocrine.com

Neurocrine Biosciences and AbbVie developed endometriosis treatment Orilissa® (elagolix), **winning FDA approval in July 2018**. Abbott, part of which became AbbVie in 2013, joined Neurocrine in 2010 for $75M upfront and up-to-$530M in milestones. "With a buyout, [AbbVie] gets full access to Orilissa without royalty and milestone fees for future indications," observed analyst Aaron Levitt in *InvestorPlace*. He cited the $409M in preliminary 2018 net product sales for Neurocrine's once-daily tardive dyskinesia treatment Ingrezza® (valbenazine), which in December failed a Phase IIb trial in Tourette syndrome.

10 Portola Pharmaceuticals

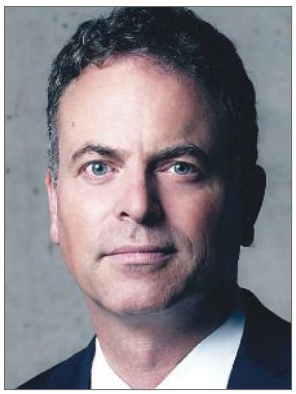
South San Francisco, CA
www.portola.com

Portola Pharmaceuticals rang out 2018 with an FDA Prior Approval Supplement for a manufacturing process **allowing for broad commercial launch of Andexxa®** [coagulation factor Xa (recombinant), inactivated-zhzo], which **won initial FDA approval in May**. It's the first and only anticoagulation reversal agent for users of Bayer/Janssen's Xarelto® (rivaroxaban) or Bristol-Myers Squibb/Pfizer's Eliquis® (apixaban) who experience life-threatening or uncontrolled bleeding. "It would be a small and logical acquisition for the large anticoagulant makers," Bret Jensen wrote January 9 in *Seeking Alpha*.

The Race Is On: What mRNA Product Will Reach the Market First?

By Ingmar Hoerr, PhD

Vaccines, enzyme replacement therapies, and cancer immunotherapies based on mRNAs vie for first-mover advantage



Ingmar Hoerr, PhD

The first "International mRNA Health Conference" was held in Tübingen, Germany, in 2013, initiated by mRNA (messenger RNA) platform companies **CureVac**, **Moderna**, and **BioNTech**. The leaders of the then-young field formed an alliance at the conference with the goal of increasing awareness of the technology. The competitors agreed to accept each other as development engines, communicate with one another, and recognize the field's co-development responsibility. Ultimately, the goal was to make a disruptive technology available to patients as quickly as possible, yet responsibly.

Now, only six years later, data is being collected in more than 25 clinical studies across numerous modalities, other companies are entering the mRNA space, the first mRNA companies are publicly listed (**Translate Bio** and Moderna), and "proof of concept" clinical studies are being initiated. Today, many eyes are on the mRNA space as the race for the first product on the market is fully underway.

What hurdles remain?

The first hurdle is the application (delivery) of mRNA. Encapsulation through packaging in lipid nanoparticles (LNPs) is currently the method of choice for most product developers. The LNP technology originates from a few companies (**Acuitas**, **Arbutus**, and **Arcturus**) and research groups (such as one composed of MIT researchers), which provided the field with licenses to bring the first mRNA projects to clinical trials. Now, some mRNA companies are developing these delivery technologies in-house. Different applications require custom LNP optimization. LNP toxicity remains a risk for multiple dosing regimens.

The second hurdle is mRNA optimization. Nature has finely regulated the half-life and expression levels of mRNA. These factors heavily influence the availability of therapeutic proteins or vaccines. It is important for vaccines to generate a high peak expression to give rise to T-cell responses and antibodies. Protein therapies, like enzyme replacement, require a high and steady expression for longer-term therapeutic benefit.

Various factors influence the expression level and half-life of mRNA, including untranslated regions (UTRs), polyA tails, cap structures, and open reading frames (ORFs). One view is that chemical modifications to the mRNA can exercise a decisive influence on the stability, immunogenicity, and expression level; another view focuses on remaining as close as possible to the natural system. As there exists a ratio of LNP required to deliver the mRNA payload, it is a significant benefit to maximize the mRNA expression levels to keep the concentration of the LNPs low for customized, highly engineered mRNA to prevent or reduce unwanted side effects.

The final hurdle is the production of mRNA. CureVac was the first company to receive GMP certification in 2005. Subsequently, other mRNA companies established production, and there are now several CMOs that offer mRNA production as a service. The quantity and quality of the mRNA is still a bottleneck. The upscaling of an industrial process with high throughput and the lowest possible production costs is a significant challenge. Molecular therapy uses, such as mRNA-coded antibodies, require large quantities, up to gram levels. The costs in the conventional production process still exceed the production costs of customary recombinant antibodies; therefore, advances in the reduction of mRNA production cost is critical.

The first product to market

The first approved mRNA product will gain a market advantage. For nearly all developers, one of the challenges is to define a product development strategy that hits big markets and exhibits the unique benefit of the mRNA application. The most lucrative markets and the greatest benefits lie in the

Ingmar Hoerr, PhD, serves as chairman of the supervisory board at CureVac. Website: www.curevac.com.

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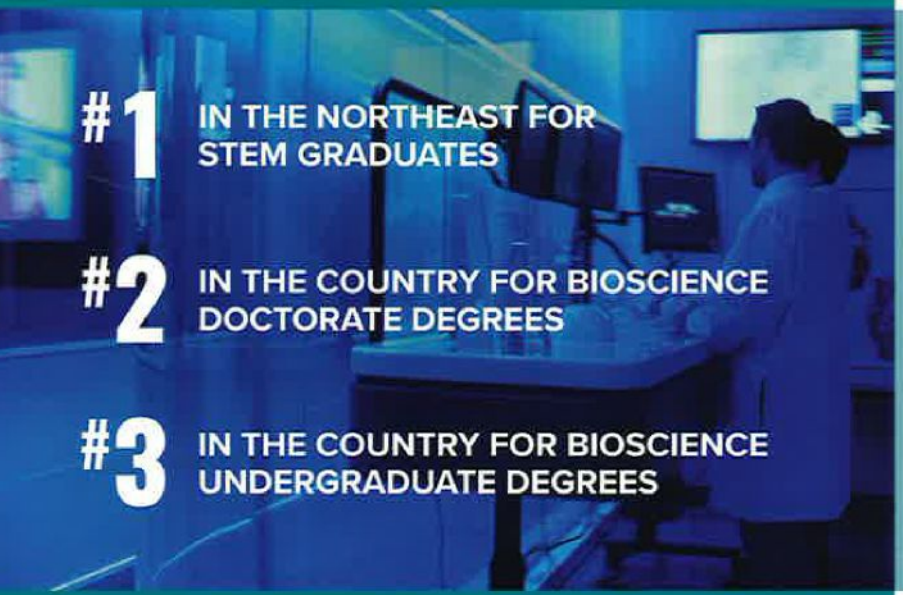

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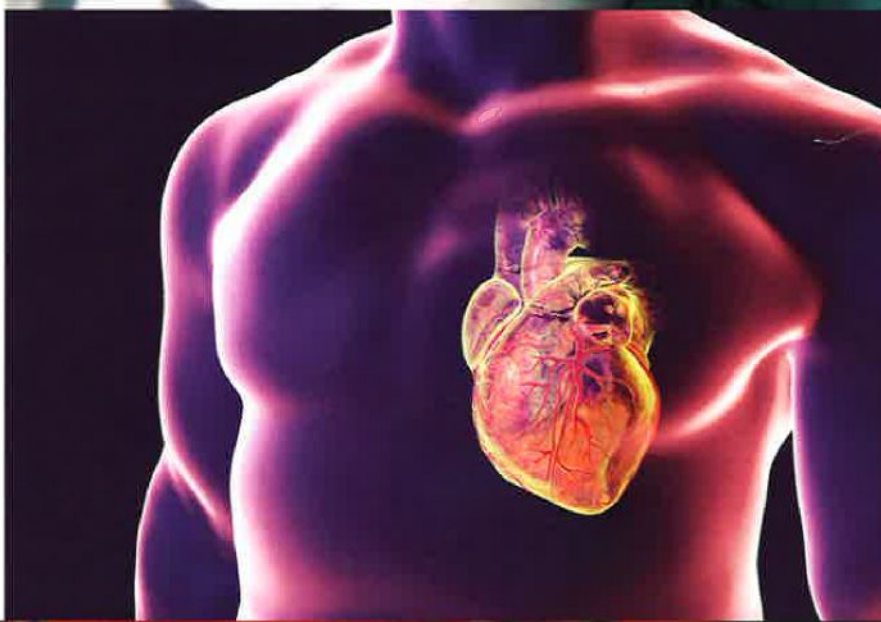
As a supplier of fully automated molecular testing solutions, Rheonix, Inc., can produce sequence-ready libraries in five hours compared to 1.5–2.5 days.

CELLULAR THERAPY: SAVING LIVES THROUGH TECHNOLOGY

University at Buffalo spin-off Cytocybernetics is advancing a drug-screening technology that integrates electronics with heart muscles to test how new drugs affect the heart's electrical activity. Such screenings could save pharmaceutical companies hundreds of millions of dollars by enabling early identification.



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expression of therapeutic proteins.

Often, mRNA-based therapies require systemic application, excellent safety, and high concentrations of the therapeutic protein, meaning high quantities of mRNA. That is why almost all developers initially chose to develop mRNA prophylactic vaccines, because small quantities of formulated mRNA via local administration is sufficient to induce an immune response. Also, the read-out based on antibody titers in the blood is standardized and quick.

Many mRNA companies have entered into partnerships to advance their broader vaccine development programs: CureVac with Bill & Melinda Gates Foundation, Translate Bio with Sanofi, Moderna with **Merck**, and BioNTech with **Pfizer**. The excellent properties of the mRNA technology enable administration of the different antigens as a cocktail. One can envision developing a broader protective vaccine including not only the most prevalent strains but multiple antigenic drifted viral isolates of one subtype. This process might be assisted by AI prediction algorithms including machine learning to better match future circulating strains. Those vaccines could be further enhanced by equipping them with conserved antigens that activate a cellular immune response.

Even if the universal vaccine remains a dream of the future, one can envision achieving at least an improved protection that lasts two to three years, which is still better than conventional market seasonal vaccines.

The second candidate for the first product to market is an enzyme replacement therapy. An in vivo bioreactor (organ) which can be efficiently loaded with mRNA, and which expresses this mRNA without causing undesired immune responses or side effects, is required. The liver is an excellent candidate for a bioreactor. It is accessible via intravenous injection of LNP-encapsulated mRNA, and LNPs effectively transfect hepatocytes.

It is only natural to turn toward liver diseases, including ornithine transcarbamylase deficiency (OTC). Many companies are already exploring this area, including CureVac/Arcturus, Moderna, **Roivant**, and Translate Bio. Local delivery to the lung is also currently under development by companies like Translate Bio, Moderna, and **Ethris**, for conditions like cystic fibrosis. Pulmonary therapies require a tight safety profile since the lung is prone to allergic and immunotoxic reactions.

The third promising candidate for the first product to market lies in oncology. Companies like CureVac, Moderna, and BioNTech are utilizing mRNA-encoded neoantigens or individual, patient-specific tumor antigens to induce systemic immune responses that target primarily metastases. Often these approaches are combined with checkpoint inhibitors.

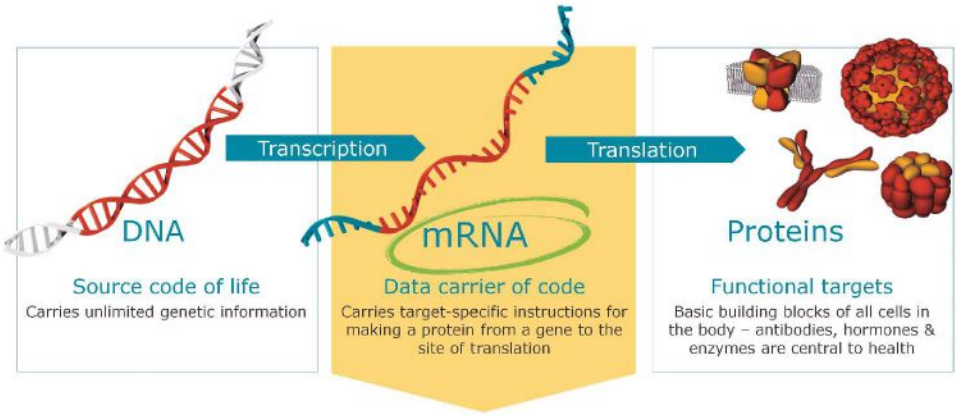
Therein lies the real magic and power of mRNA technology: It creates a universe of new treatment options.

Another approach to generate a local effect of RNA is the direct application of RNA in the tumor. The aim is to put pressure on the tumor by injecting immunostimulating RNA- or mRNA-encoded immunomudulators. This process turns "cold" tumors "hot," introduces apoptotic cell death, leads to a release of tumor-associated antigens, and triggers a systemic immune response, which can target even nontreated tumor lesions.

The ultimate goal of this approach is to have cancerous cells act as their own vaccine. In the field of intratumoral application, there is currently a gold-rush atmosphere; there are several other technologies, such as oncolytic virus constructs, which make use of a similar principle of action. CureVac, Moderna, and BioNTech are exploring various mRNA compositions.

Conclusion

There is an exciting race to bring the first mRNA therapy to market. All mRNA competitors know that this product will have a signaling effect on all future product developments. This effect is needed to develop new disruptive approaches that include completely new mechanisms of action. For example, mRNA technology can be used to influence intracellular pathways or to express membrane receptors, opening new therapeutic possibilities. Therein lies the real magic and power of mRNA technology: It creates a universe of new treatment options. Once one mRNA medicine enters the market, many more will enter the clinic. The possibilities are truly limitless. GEN



CureVac notes that its mRNA therapeutics follow the same functional principles found in nature. When supplied the right mRNA, the body can produce its own medicinal proteins.

GEN OMICS

Incomplete Genomics:

Adding New Sequences to the Reference Genome

By Julianna LeMieux, PhD

Illustration by Mon Oo Yee

Sequencing experts fill in gaps, confront genomic diversity, and ponder the complexities posed by multiple reference genomes

On April 14, 2003, the National Institutes of Health issued a press release announcing that the Human Genome Project had been completed ahead of schedule and under budget. At the time, the human genome was considered, to all intents and purposes, complete. Almost 16 years later, as of the writing of this article, www.genome.gov, a website run by the National Human Genome Research Institute, responded in the affirmative to the question, "Is the human genome completely sequenced?" The "Yes," it must be said, was followed by a hedged statement: "Within the limits of today's technology, the human genome is as complete as it can be."

Over the years, it has become increasingly evident that the reference genome is not the pristine, essentially complete human genome sequence one might believe. Researchers such as George Church, PhD, professor of genetics at Harvard Medical School, have pointed out that stubborn hard-to-sequence portions of the human genome almost certainly contain medically relevant genes. Also, a report published in early 2018 used nanopore sequencing to define the previously uncharted DNA from the centromere of the human Y chromosome. But the biggest surprise was published late last year.

A group led by Steven L. Salzberg, PhD, professor of biomedical engineering, computer science, and biostatistics at Johns Hopkins University (JHU) Medical School, reported in *Nature Genetics* that a trove of DNA sequence information is missing from the reference genome. The group's analysis of a dataset of 910 individuals of African descent revealed that the reference genome omits roughly 300 million base pairs (or megabases, Mb)—almost 10% of the entire reference genome.

The utility of the reference genome in advancing genomics over the past 15 years is not in question. To think that its purpose is simply for reanalyzing other genomes is myopic. It is the coordinate system that is used for annotation. It has enabled rare disease research and furthered genome sequencing and assembly work. In addition, large-scale genomic inventories, such as ENCODE (the Encyclopedia of DNA Elements, a public research effort that has identified a wealth of functional elements in the human and mouse genomes) and the 1000 Genomes Project (an international research effort to establish the most detailed catalog of human genetic variation), would have been impossible without the human reference genome.

The missing DNA

Salzberg and colleagues, led by graduate student Rachel Sherman, began their project by examining DNA that had been collected from 910 individuals by the Consortium on Asthma among African-Ancestry Populations in the Americas (CAAPA). Salzberg tells *GEN* that he had been looking for a unique resource like CAAPA and was lucky that his colleague Kathleen Barnes, PhD, who moved to the University of Colorado Anschutz Medical Campus after 23 years at JHU, was studying asthma and allergy in the African population where those conditions appear at a higher background rate.

Rachel Sherman

The utility of the reference genome in advancing genomics over the past 15 years is not in question.

The CAAPA dataset contains 1000 genomes, each with more than 1 billion reads, collected from people who live around the world, including the United States, Central Africa, and the Caribbean. It is well known that African populations—comprising more than 2,000 distinct ethno-linguistic groups—exhibit a greater degree of genetic diversity than non-African populations.

Sherman aligned a total of 1.19 trillion reads from the CAAPA individuals to the reference genome (GRCh38) to construct what she calls a pan-African genome. The results were published in the November 2018 *Nature Genetics* paper, "Assembly of a pan-genome from deep sequencing of 910 humans of African descent." Most of the novel DNA fragments were 1000–5000 base pairs long, with the largest chunk being 152,000-base-pairs long. However, both the functional significance of the sequences and their locations remain mostly unknown.

Some sequences had their locations determined. These sequences, Sherman tells *GEN*, appeared to be fairly randomly distributed, and inserted sequences were found in 315 genes. Sherman adds that a good portion of the sequences are likely to be in the centromeres and telomeres because those regions are less well represented in the reference genome than other sequence.

Salzberg is no stranger when it comes to human genome analysis. From 1997 to 2005, he was on the faculty of The Institute for Genomics Research (TIGR), the nonprofit set up by J. Craig Venter, PhD, and was a co-author on the **Celera Genomics** draft human genome report in 2001. But Salzberg was astounded by the main result of his latest

study. He had predicted his team would uncover perhaps 8–40 Mb of novel DNA, but never expected to find 300 Mb. Of course, Salzberg's team considered the presence of contamination and has reanalyzed the data in multiple ways to ensure that this is a bona fide result.

According to Deanna M. Church, PhD, currently senior director of mammalian applications at **Inscripta**, who worked for more than 10 years at the National Center for Biotechnology Information and was closely involved in building the human reference genome, the identification of novel human genome sequences not seen in the reference is not so surprising. Although Salzberg used short-read sequencing—specifically, **Illumina** sequencing—other researchers have used other approaches, such as long-read technology, to describe novel sequences.

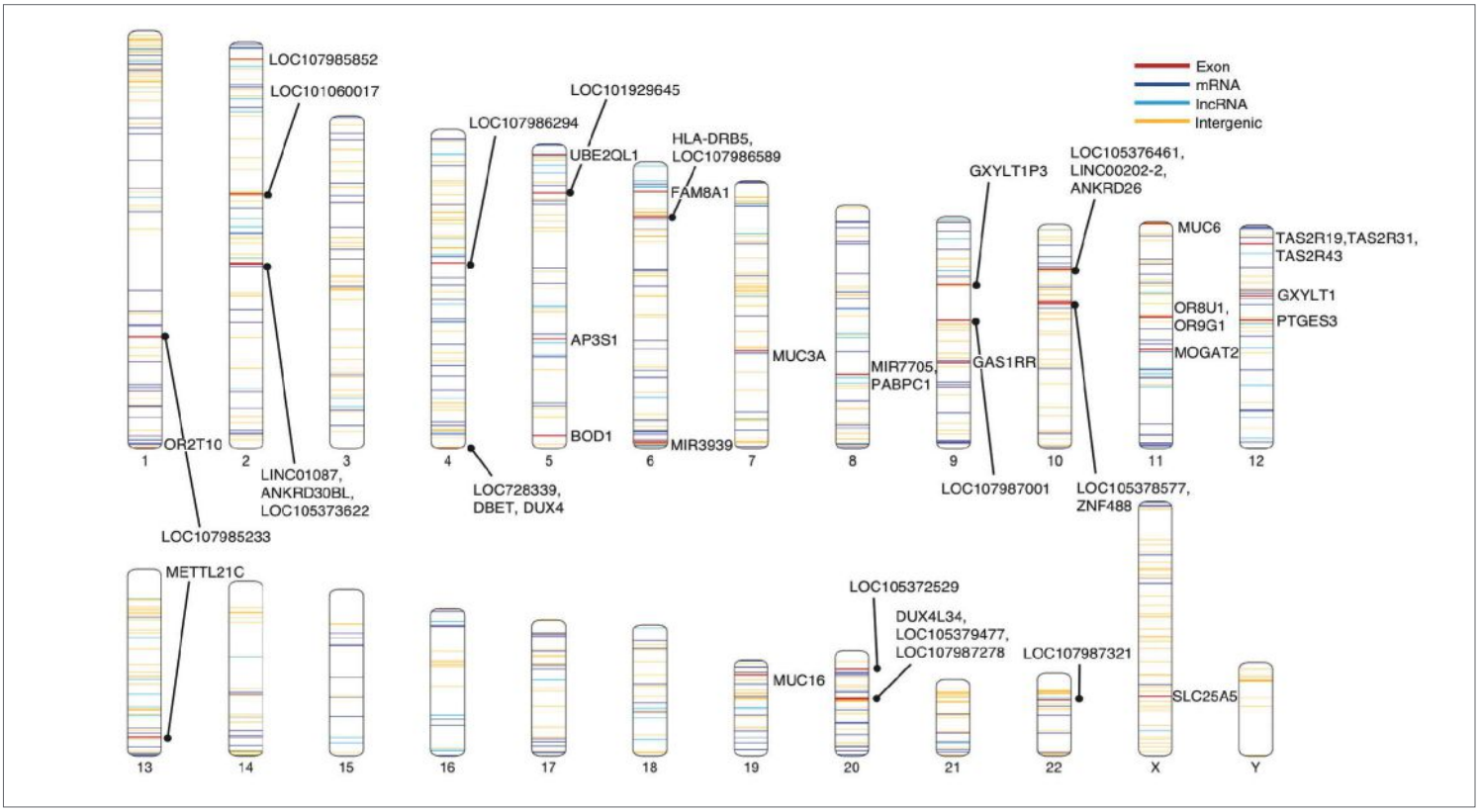
Deanna M. Church, PhD

The "300 Mb" figure needs to be taken with caution, Church says, because the technical issues that accompany assembly methods in short-read technology cannot distinguish between haplotypes in the donor samples. As a result, sequences might be created that don't exist in the population because the haplotypes get mixed in ways that don't make sense. When that happens, technical artifacts are created that don't align with the reference. That is, some of the sequences that are classified as novel might instead be located in areas where there is a lot of haplotypic diversity.

Church recalls an experiment she was involved in when updating the previous GRCh37 assembly to the current GRCh38 assembly. Because Church and colleagues were adding sequences into the GRCh38 reference, they knew that the sequences they were working with were not already present. So, these sequences could be thought of as equivalent to Salzberg's missing sequences.

When Church and colleagues cut them down and aligned them to the reference, roughly 70% of these reads aligned to the GRCh37 reference using multiple alignment methods." Church says this shows that, "just because a sequence isn't represented in the reference doesn't mean that it won't align to the reference." She adds that follow-up experiments with technology that allows the separation of sequences into haplotypes will yield more robust data.

When the reference genome was made, two haplotypes were mixed together to make a mosaic consensus. Today, however,



A map of the human genome showing the locations of the African pan-genome contigs whose locations could be identified. The elements were located on every chromosome and dispersed throughout the genome. All insertions that are within an exon are labeled with the gene name. Reprinted from Sherman RM, Forman J, Antonescu V, et al. *Nature Genetics* 51: 30–35 (2019).

new technology is available that makes haplotype separation possible, and some groups, such as the 1000 Genomes Project's structural variation (SV) working group, are starting to focus on this aspect of genomic analysis.

One method that can separate haplotypes is long-read sequencing, like the sequencing technology developed by **Pacific Biosciences** (which is currently undergoing an acquisition by Illumina) and **Oxford Nanopore Technologies**. A single read comes from a single molecule of DNA, which will be a single haplotype. With multiple long reads, haplotypes can likely be separated because there are enough haplotype-specific single nucleotide polymorphisms (SNPs) that overlap between reads.

Another method is "linked reads," a new sequencing technology developed by **10X Genomics** in which multiple short reads from the same molecule (same haplotype) are tagged with the same barcode. Therefore, it is known that they came from the same haplotype.

Yet another method is Hi-C based scaffolding. It is being developed by companies such as **Dovetail Genomics** and **Phase Genomics** to determine 3D chromosome structure. These relatively new techniques can be used together as well.

Genomic diversity

Salzberg opines that the lack of diversity in the reference genome is a problem, the size of which "depends on what you're using the reference genome for." Much of what we use it for is to determine genetic causes of disease and/or cancer. And so long as researchers are studying a person with a similar genetic background to the reference, using the reference genome this way is fine. But "at least when looking at the African pan-genome, there is quite a lot of variation that's missing."

Geneticist Nathaniel Pearson, PhD, the founder of **Root**, a company that rewards blood and marrow donor volunteers with insight from their own HLA genes, agrees that the reference genome has limitations. "Since we use the reference genome for several different things, and we've long been using a single reference genome to try to do all of them, [it] ends up doing none of them ideally," Pearson says.

Documenting the extent of human genome variation is an ongoing challenge. For years, the genomics community relied on SNP analysis because that is what the technology allowed. But over the past decade, researchers have cataloged large chunks of DNA that are present or absent in different people—so-called copy number variations. A great deal of the genomic variation in the human population may reside in these larger structural variations—insertions and deletions ("indels") of thousands or tens of thousands of bases. As Salzberg says, to fully understand those differences, you need to sequence more genomes.

> ***"Before the Human Genome Project started, our understanding of diversity in the population was very limited and simple."*** —Deanna M. Church, PhD

For future reference

What, then, is the fate of the reference genome? Salzberg argues in the *Nature Genetics* study that "a single reference genome is not adequate for population-based studies of human genetics. Instead, a better approach may be to create reference genomes for all distinct human populations, which will eventually yield a comprehensive pan-genome capturing all of the DNA present in humans." He tells *GEN* that making reference genomes for distinct human populations' genomes is not only the best solution, but that it will likely be the solution. It is, says Salzberg, "just a matter of time."

Salzberg believes that for a study of the genetic propensity of any disorder, researchers should have a reference genome "from a normal, healthy individual from that population." But what is a normal, healthy person? Pearson thinks it is a fool's errand to try to make a "healthy only" reference genome for many reasons. For example, many genetic variants have varying penetrance, and a DNA donor could be healthy at the time of donation, only later to be diagnosed with cancer.

Deanna Church says that a solution is more complicated than just making more reference genomes, but fully supports collecting more sequences from diverse populations. However, admixture (the introduction of new genetic lineages into a population) is another complication to consider. If an African American is 50% admix European and West African, which reference genome would be most appropriate to use?

Another hurdle is determining how to efficiently compare a sequence query to a large set of diverse reference genomes. Salzberg and his former student and current JHU colleague Ben Langmead, PhD, an assistant professor of computer science, developed Bowtie in 2009—an algorithm used to align large quantities of shotgun sequencing data to the reference genome.

Rather than think in terms of a simple linear reference genome, Church says that many genomics researchers favor something called a variation graph representation. Variation graphs are DNA sequence graphs that represent genetic variation, including large-scale structural variation.

Last summer, Erik Garrison, a PhD student in the lab of Richard M. Durbin, PhD, at the Wellcome Trust Sanger Institute in Cambridge, UK, published a multipurpose toolkit in *Nature Biotechnology* of computa-

tional methods to perform genomic analysis using a variation graph as a reference named "vg." This approach is the beginning of people having the tools necessary to do these analyses on their own. But not everyone thinks that the graph structure will succeed, and Church admits that it is still too early to see if the graph approach will catch on.

To solve the limitations of the reference genome, Pearson's preference would be to create an ancestral reference genome. "At every spot on every chromosome, the letter that we [would] write at that spot is the letter shared by the last common ancestor of all the human copies today," Pearson explains.

In principle, this would create a universal coordinate system for every person's genome. An ancestral reference genome, Pearson continues, has several advantages: it is ethnically equitable, allows the inference of whether a piece of DNA is an insertion or a deletion, and removes the issue of the ideal health status of reference genome donors. Salzberg says the approach is interesting from an evolutionary point of view, but of limited value, he argues, for understanding the genetic causes of disease.

With new sequencing technology and analysis software, filling in the missing gaps in the reference genome will be easier. For example, in October 2018, Pacific Biosciences unveiled the most contiguous diploid human genome assembly of a single individual to date. Pearson notes that the best reference genome for any given person is their own. With the falling cost of genomic sequencing, perhaps the day is coming where our reliance on a reference genome will no longer exist. GEN

Whose DNA Makes Up the Reference Genome?

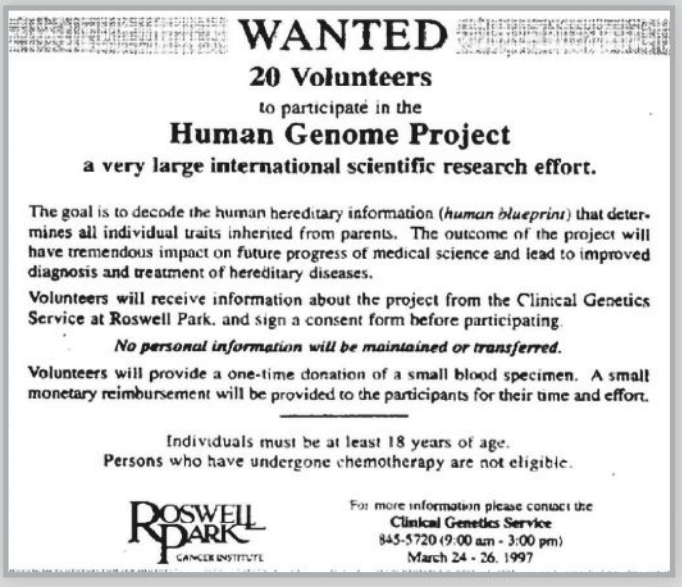
In early 1997, master DNA library builder Pieter J. De Jong, PhD, placed an ad in a Buffalo, NY, newspaper seeking volunteers for the Human Genome Project. These anonymous genome pioneers provided the DNA from which the reference genome was assembled a few years later. At about the same time, J. Craig Venter, PhD, and colleagues at Celera Genomics assembled their own genome from five major donors of different ethnic backgrounds, including (it was later revealed on national television) Venter himself.

In retrospect, the Human Genome Project's recruitment strategy seems insufficient. "Before the human genome project started, our understanding of diversity in the population was very limited and simple," Deanna M. Church, PhD, tells *GEN*.

The makeup of the reference genome was not published at the time it was released, so the origin of the DNA was anyone's guess. In subsequent years, the guesses ventured likely reflected the awareness that genome-wide association studies (GWAS) are primarily conducted on European populations. Recent analysis indicates that although the reference genome is composed of DNA from several different contributors, the largest single donor was a single African-American individual, known as RP-11.

The first formal acknowledgement of this result did not appear in the literature until the release of the Neandertal genome. Appearing May 2010 in an article published in *Science*, this genome resulted from work led by Svante Pääbo, PhD, an evolutionary geneticist at the Max Planck Institute for Evolutionary Anthropology, and David Reich, PhD, a geneticist at Harvard Medical School. Buried in the article's 150-page-long supplement was an analysis indicating that about 70% of the reference genome was contributed by RP-11, an individual who appeared to be of half African, half European descent. (That same report also noted that a small percentage of the reference came from a person of 100% Chinese descent.)

The strategy used to compile the reference genome wouldn't suffice today. A new strategy would likely reflect what has been learned since the completion of the Human Genome Project, including, Church suggests, a different approach to consent. Of course, now that direct-to-consumer genetic testing kits are available, the notion of genetic privacy has changed completely.Ideally, Church adds that future reference genome projects would take extensive health information from diverse donors. ■



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An ad that appeared March 23, 1997, in the *Buffalo News* helped launch the Human Genome Project. Placed by Pieter J. De Jong, PhD, the ad asked volunteers to provide blood samples from which DNA could be extracted.

Tumor Microenvironments Are Target-Rich Environments

By Vivienne Raper, PhD

Anticancer strategies equip the immune system to recognize (and rout) well-camouflaged foes

Pharmaceutical companies are investigating new lines of attack against cancer, which is, according to the World Health Organization, the world's second leading cause of death. In 2018 alone, cancer accounted for 9.6 million deaths across the globe, or about 1 out of every 6 deaths that year. If cancer statistics are to become less daunting, it may be necessary, many pharmaceutical companies believe, to explore alternatives to traditional anticancer approaches.

Cancer research has focused on the disease's underlying genetic and epigenetic defects. Today, however, pharmaceutical companies are turning their attention to the tumor microenvironment (TME), a battleground that consists of the elements surrounding cancerous cells. It includes specialist immune cells, vascular cells, and other factors that contribute to cancer progression.

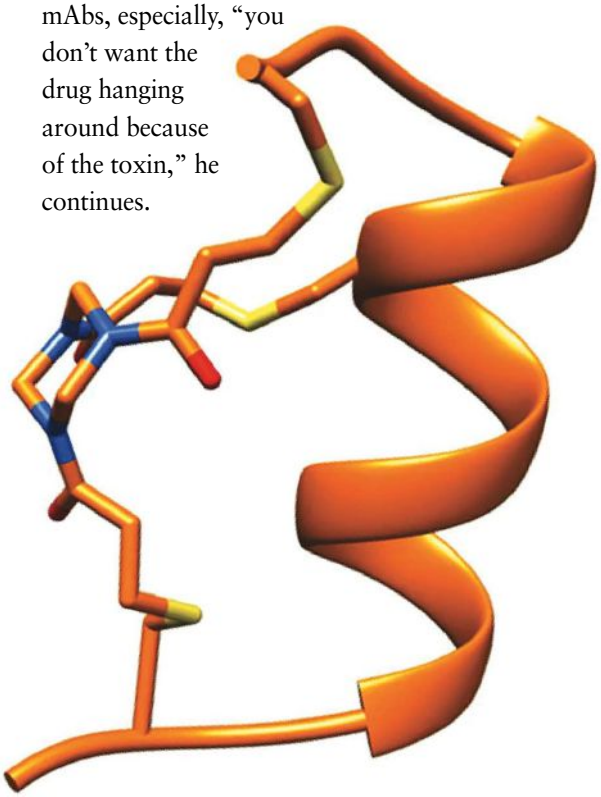
On this battleground, the fog of war may confound the immune system, preventing it from distinguishing friend and foe. Dispelling the fog, then, could help the immune system fight more effectively. For example, the immune system could gain situational awareness via immunotherapy. Although currently available immunotherapies benefit only a minority of cancer patients and often cause side effects, newly developed immunotherapies may perform better—provided they start targeting the TME.

TME-targeting possibilities were discussed at the 10th Annual Protein and Antibody Engineering Summit (PEGS) Europe. This event, which took place in Lisbon, Portugal, last November, included an immunotherapy track entitled, "Targeting the Tumor Microenvironment." Several presentations from this track are highlighted in this article, including presentations on bicycle peptides; combination therapies; oncolytic viruses; antagonistic antibodies for killing both tumor cells and immunosuppressive T cells; and improved checkpoint inhibition strategies.

First on a bicycle

Monoclonal antibodies (mAbs) are the commonest type of immunotherapy. These are antibodies grown in cloned immune cells, which target a specific antigen expressed by a tumor. mAbs for immunotherapy have many different mechanisms of action. Some mAbs, for example, flag the tumor for destruction by immune cells, whereas others block antigens that help the tumor grow and spread. Conjoined mAbs, meanwhile, bond to toxins or radioactive particles and deliver them directly to the tumor.

Problems posed by mAbs include their large size and long half-life within the body, says Kevin Lee, PhD, CEO of **Bicycle Therapeutics**. With conjoined mAbs, especially, "you don't want the drug hanging around because of the toxin," he continues.



Bicycle Therapeutics' Bicycles can adopt a range of biologically important conformations, such as alpha helices, that mimic sites of protein–protein interactions.

When multiple long-duration antibodies are used in a combination immunotherapy, they can lead to the overactivation of the immune system with consequent side effects and a loss of efficacy because "you've got the foot on the accelerator of a car all the time."

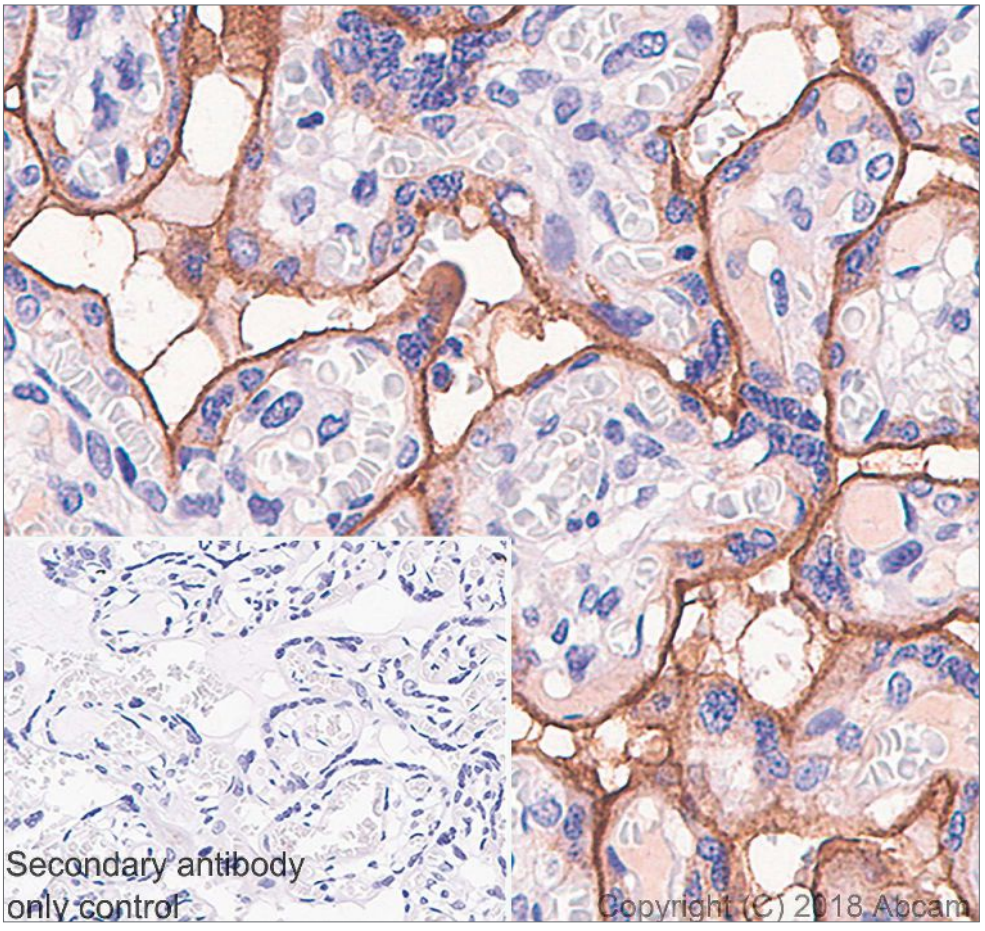
Bicycle Therapeutics is pioneering a new class of immunotherapies based on bicycle peptides, synthetic peptides between 9 and 15 amino acids in size that are tied to a small central molecular scaffold. "I think we have something very different, disruptive, and transformational in the field," Lee asserts.

Bicycle peptides were invented by Sir Gregory P. Winter, PhD, a member of the MRC Laboratory of Molecular Biology in Cambridge, UK, and a winner of the 2018 Nobel Prize for Chemistry. At PEGS Europe, Winter spoke as the director of Bicycle Therapeutics. "Greg spent a long time working on the minimal chemical footprint of an antibody needed to replicate some of its activities," Lee points out, emphasizing that bicycle peptides are much smaller than antibodies and capable of rapidly penetrating tumors. Bicycle peptides, then, may exert their effects without having to rely on long circulating half-lives in the body.

The company has a lead compound currently in Phase I testing. This consists of a bicycle bonded to a tumor-killing toxin. Whereas antibodies bonded to toxins kill a tumor as though they were "peeling an onion layer by layer," Lee explains, a diminutive Bicycle Toxin Conjugate (BTC) can "blow up the tumor from the inside" like the well-targeted torpedo that entered and destroyed the Death Star in *Star Wars*.

After validating its technology, Bicycle Therapeutics hopes to move two additional BTCs into the clinic. BT5528 targets the ephrin type-A tumor antigen, whereas BT8009 targets the Nectin-4 tumor antigen. The company is also developing a bispecific bicycle that activates T cells to destroy cancers.

In addition, Bicycle Therapeutics is working on a STING (stimulator of interferon genes protein) agonist. STING agonists stimulate the release of interferon gamma, a signaling molecule that upregulates the innate immune system. Lee observes that STING agonists are generating a lot of pharmaceutical interest even though current treatments tend to activate multiple immune cells, causing major side effects. He hopes that a bicycle with a cleavage linker will be developed that stays attached to a STING agonist while the bicycle guides the agonist into a tumor, and that releases the agonist only *after* it is inside.



Abcam scientists subjected formalin/paraformaldehyde-fixed paraffin-embedded sections of human tonsil to immunohistochemistry (IHC) analysis. To detect PD-L1, the scientists used Abcam's PD-L1 RabMAb antibody (clone 73-10, ab228415) at 1/500 dilution. The secondary antibody came from the company's ready-to-use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Benefitting a majority

Combination therapies tailored to patients are a major trend in targeting the TME, says Jamie Campbell, head of custom services, **Abcam**. "I think single therapies are not going to be the norm. Duplicate or triplicate therapies will be the norm in future, so having good quality reagents is the core of showing those expensive drugs are performing as expected," he explains. Abcam provides reagents and tools for research, drug discovery, and diagnostics to pharmaceutical companies targeting the tumor microenvironment.

Another trend he identifies is customers wanting reagents that act as indicators of whether a patient group will benefit from a specific therapy. "There's a trend in the

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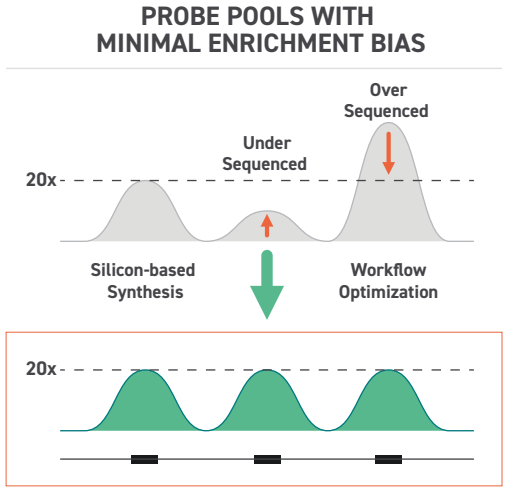

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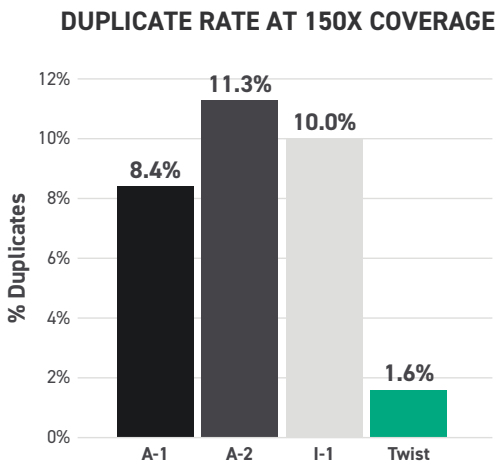

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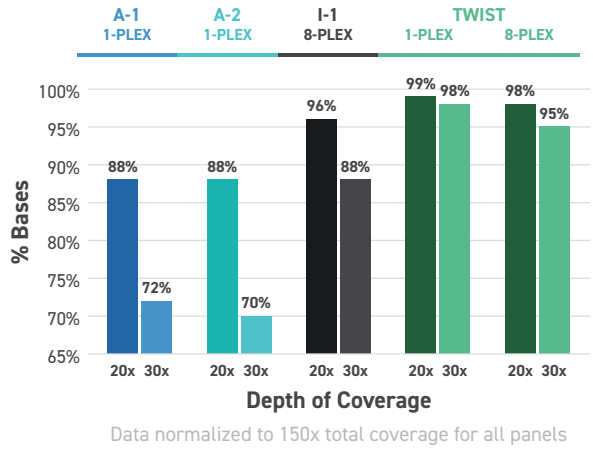

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industry to show that new drugs will be effective," he elaborates, "because antibody- and protein-based therapies are more expensive than small molecule approaches."

A significant part of reason these trends exist is the limited success of the first generation of cancer immunotherapies, that is, checkpoint inhibition and chimeric antigen receptor (CAR) therapies. Initially, these immunotherapies generated a great deal of enthusiasm. For example, in 2013, cancer immunotherapy was awarded Breakthrough of the Year by the journal *Science*. Interest in cancer immunotherapy remains high, but developers are increasingly aware of challenges including side effects, partial responses, and lack of efficacy in most patients.

Cancer vaccines

"As we have more knowledge about checkpoint inhibition, we understand that it's not a therapy for everyone. It benefits in the range of 10% of patients, depending on the tumor indication, but there's still a majority who aren't taking a major benefit," said Philipp Mueller, PhD, principal scientist for cancer immunology and immune modulation, **Boehringer Ingelheim**.

Boehringer Ingelheim is conducting preclinical testing of an oncolytic virus, a vesicular stomatitis virus carrying a modified glycoprotein, that is, a VSV-GP. This VSV-GP selectively targets cancer cells, and the company hopes that it will benefit patients who don't respond to checkpoint therapies. These patients often have so-called cold tumors, which don't have T-cell inflammation, and therefore can't be treated by checkpoint inhibitors.

"Cancer cells have deficits in their innate immune response pathways and are unable to protect themselves from a viral infection," Mueller explains. The aim of the viral therapy is for the virus to selectively penetrate and attack the tumor cells without affecting healthy tissue, inducing the patient's immune system to activate against the tumor.

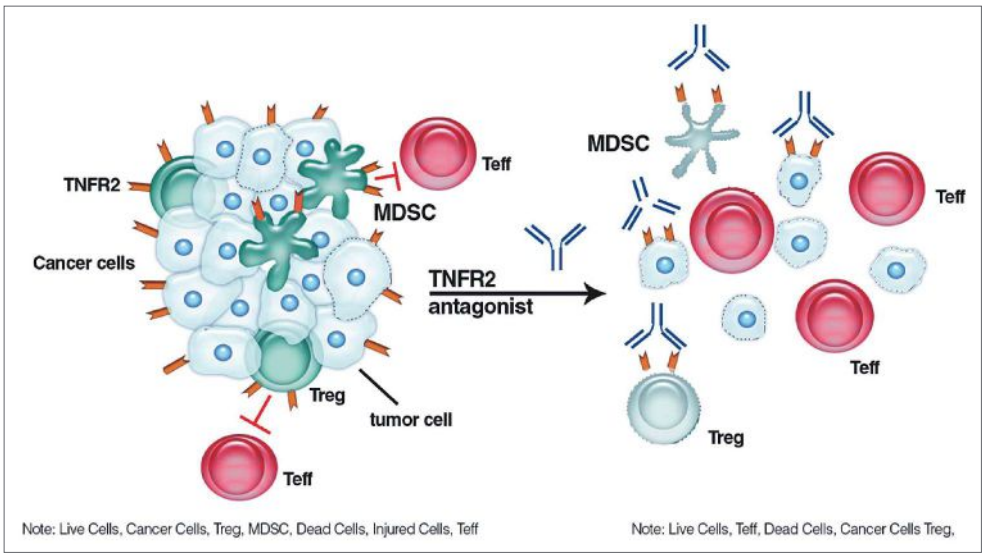
Oncolytic viruses aren't new, but the hope is that VSV-GP will improve upon existing therapies. "The oncolytic viruses currently available as a product or next-stage development are frequently based on big DNA viruses; the benefit of this small virus is that it's able to replicate very fast," says Knut Elbers, PhD, CEO of ViraTherapeutics, all shares of which were bought by Boehringer Ingelheim in September 2018. VSV belongs to the class of rhabdoviruses which, Elbers explains, can replicate in as little as eight hours. He believes this makes it a powerful tool for fast and effective activation of the immune system, making it ideal for use in combination with other therapies.

> ***"Cancer cells have deficits in their innate immune response pathways and are unable to protect themselves from a viral infection."***
>
> —PHILIPP MUELLER, PhD

Working in combination

Denise L. Faustman, MD, PhD, director of immunobiology at Massachusetts General Hospital, is developing an antibody therapy that works to help patients who fail to respond to checkpoint inhibitors. "If you look at data on checkpoint inhibitor failures," she says, "you see that when those checkpoints fail therapeutically, the dominant protein on the regulatory T cells (Tregs) is tumor necrosis factor receptor 2 (TNFR2)." When TNRF is expressed on Tregs, which are T cells that regulate or suppress other parts of the immune system, the Tregs become super-suppressive.

TNFR2 is found on the surface of a tiny fraction of the most immunosuppressive Tregs in healthy people, but is found in large quantities in the microenvironment of some tumors. "In all cancer targets to date, I've never seen one that's preferentially expressed in the TME and acts both as a T-cell suppressor and an oncogene, and which has low expression in the human body," she points out.



Tumor necrosis factor receptor 2 (TNFR2) promotes direct tumor proliferation through oncogene expression or through overexpression on tumor-residing regulatory T cells and myeloid suppressor cells. According to Massachusetts General Hospital researchers led by Denise Faustman, PhD, targeted alterations in the tumor microenvironment may be possible with multifunctional TNFR2 antagonistic antibodies.

"In TNFR2," she continues, "you've got a protein that is a bad boy in the TME in two limbs: It's over-expressed on Tregs in the TME, making them super-suppressive, and TNFR2 is also a proliferative pathway, thus also expanding the cells preventing effective therapies—that's what makes it so different." After 10 years of work, she has an antibody in preclinical testing that preferentially binds TNFR2 on the surface of proliferating Tregs in the TME, killing these cells while leaving the body's natural immune response intact.

She hopes that her new therapy will be ready for testing in humans in 12–18 months, and that it will prove useful in combination with other treatments. As TNFR2 is expressed in low concentrations on the surface of normal cells, she hopes it will have fewer side effects than existing therapies. "There's been a lot of progress in targeting checkpoints," she notes, "but there's a lot of toxicity, so a better toxicity profile is a beautiful thing."

Making checkpoints better

Among the most popular checkpoint inhibitors are those targeting programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1). According to the Cancer Research Institute in 2017, there were more than 1500 clinical trials underway involving PD-1/L1. Researchers at **Pfizer** are developing a mouse model to aid studies into how patients respond to anti-PD1/PD-L1 therapies.

"One challenge any preclinical researcher faces is whether the murine model will be relevant and translatable to human patients in clinic," indicates Yan Qu, PhD, senior principal scientist at Pfizer. Her aim is to understand why some patients don't respond to anti-PD1/PD-L1 therapies and immune activity in the TME during drug treatment. She hopes to gain insights that may lead to more efficient drugs combination therapies.

Qu is seeking to understand the signaling pathways and immune signatures within the TME that lead to the therapies failing. "Just like what has been observed in clinic, we have observed that some preclinical murine models mimic human patients' response to anti-PD1/PD-L1 therapy," she notes. "Although these mice are an inbred strain (which means they're genetically identical), their response to PD-1/PD-L1 blockade are heterogenous." She is hoping to use data from clinical trials to validate the model. GEN

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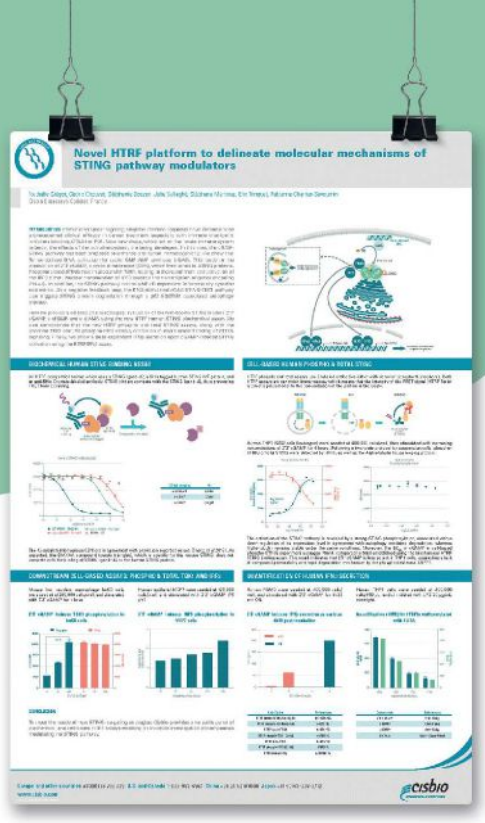

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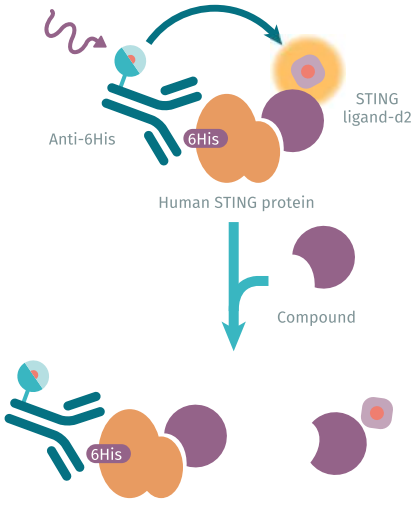

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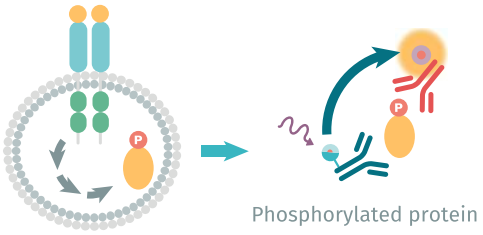

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Epitope Mapping Sees around Corners

By Richard A. Stein, MD, PhD

Antigenic forms that lie in wait can be flushed out by sequencing and display technologies, or espied by high-resolution imaging

Antigenic forms of high interest often keep a low profile, frustrating investigators who would like to identify and characterize epitopes, the parts of antigens that are recognized by antibodies. Epitopes can hide in discontinuous amino acid sequences, lurk behind strategically placed protein folds, or simply seek safety in numbers. To get around these evasion strategies, investigators may take advantage of sophisticated technologies, including protein engineering, imaging analyses, and molecular modeling. Using these technologies to identify the molecular and structural determinants of antibody–antigen interactions can help investigators advance rational drug and vaccine design.

Newly available technologies, it should be emphasized, are improving epitope mapping in both spatial and temporal dimensions. That is, they help investigators characterize a dynamic antibody–antigen landscape. Just as satellite imaging of a war zone can reveal shifting patterns of activity, epitope mapping can show the sequence changes that unfold as disease progresses. In either case, when evolving threats are tracked, it is easier to design and deploy powerful countermeasures.

Masking superfluous epitopes

"In vaccine design, one of the newest ideas is to 'epitope focus' the immune response," says Jonathan R. Lai, PhD, professor of biochemistry, Albert Einstein College of Medicine. There are several ways to accomplish that, and the approach used in Lai's lab, known as protein resurfacing, involves protein engineering to change solvent-exposed regions.

A major effort in Lai's lab is pursuing novel dengue vaccination strategies. There are four naturally occurring dengue virus serotypes. While the initial infection is usually a self-limited febrile illness, secondary infection with a dengue virus of a different serotype leads to severe and life-threatening manifestations, including hemorrhagic fever and shock. This is thought to occur when pre-existing antibodies enhance the infection with a heterologous serotype rather than protect against it.

Developing broad and potent antibody responses is a critical component of novel vaccination strategies. To address this need, Lai and colleagues have sought ways to develop vaccines that provide broad protection against all four co-circulating serotypes.

Previously, Lai and colleagues characterized the recognition between domain III of the envelope glycoprotein, an attractive viral immunogen, and 4E11, one of the broadly neutralizing antibodies.

"We structurally engineered domain III from dengue virus 2 so that we could 'immune mask' portions of the domain," indicates Lai. This approach prevented antibody responses to the portions the investigators wanted to overlook, but it maintained the portions the investigators wanted to target. Using mutagenesis selection, Lai and colleagues generated domain III variants in which the main epitope to protect was maintained but other epitopes were removed.

The construct led to a cross-neutralizing response for dengue virus serotypes 1, 2, and 3, something that has not been achieved before with single-domain constructs. "We are trying to develop approaches to improve the strength of the immune response and to extend the coverage to serotype 4," says Lai.

While protein engineering has expanded in recent years, it is still difficult to predict the immune response to newly generated epitopes. "There is a lot of potential for this approach to find new vaccine candidates," says Lai, "but it will take a long time to figure out all the rules and before this becomes a mainstream approach for discovery."

Displaying crucial folds and residues

"We can rapidly engineer our yeast platforms to make sure that proteins look the way they do naturally," asserts Timothy A. Whitehead, PhD, associate professor of chemical and biological engineering, University of Colorado Boulder. "We can easily do this for a number of antibody candidates."

In a recent study, Whitehead and colleagues used a combination of yeast surface display, saturation mutagenesis,

Detailing the molecular and structural determinants of antibody–antigen interactions can help investigators advance rational drug and vaccine design.
Alfred Pasieka / Getty Images

At Left: jxfzsy / Getty Images

fluorescence-activated cell sorting, and deep sequencing to identify pro-region mutations that enhanced the folding of nerve growth factor, a protein that plays a central role in multiple chronic pain conditions. From over 1700 point mutants generated in an analysis of canine nerve growth factor, Whitehead and colleagues identified several candidates that showed a markedly improved folding. This approach demonstrated the possibility to understand the contribution of individual amino acids to antibody recognition and neutralization.

In another research effort, Whitehead and colleagues sought to better understand the individual antibodies elicited by the acellular pertussis (whooping cough) vaccine. The antibody titers decline somewhat faster for this vaccine than for many other vaccines or infectious diseases.

Whitehead and colleagues isolated antibody sequences from individual plasmablasts, and the investigators identified, for the first time, human antibodies stimulated by the vaccine. Using a combination of mutagenesis and yeast display, the investigators generated a detailed map of the epitopes that are recognized by two of these antibodies. This epitope mapping strategy enabled the investigators to devise a structural explanation for the inability of one of the antibodies to neutralize the pertussis holotoxin.

Epitope mapping of immune responses to specific antigens can achieve more detailed immune profiling.

More generally, this strategy emerges as a promising experimental approach to elucidate the mechanisms of protection and waning protective immunity during disease and vaccination. "We would like to see epitope mapping improve and move upstream," declares Whitehead. "For these technical challenges, we need a way to do sequencing a little cheaper and faster."

Shadowing shape-shifting biomarkers

"Our hypothesis is that high-resolution epitope mapping of the immune response against specific relevant proteins will identify particular immune phenotypes and help us better understand, diagnose, and eventually treat certain conditions," says Armin Alaedini, PhD, professor of medicine, Institute of Human Nutrition, Columbia University Medical Center. A major effort in Alaedini's lab is using translational research to dissect disease mechanisms and identify biomarkers.

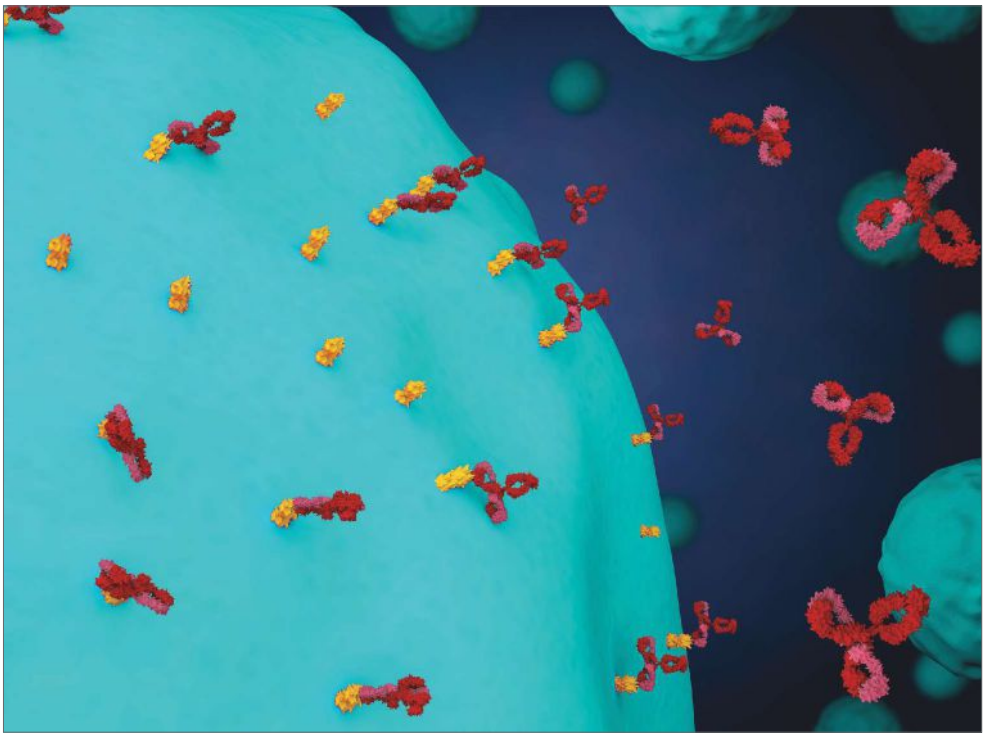
"We are interested in disorders that are generally understudied and for which no established biomarkers exist," says Alaedini. Conditions that Alaedini's laboratory are currently focused on include autism spectrum disorder, irritable bowel syndrome, chronic fatigue syndrome, and post-treatment Lyme disease syndrome (PTLDS).

"The etiologies and mechanisms of these conditions remain unclear," Alaedini observes. "But a number of studies indicate they are all associated with immune abnormalities, as demonstrated by changes or differences in the immune response of affected individuals, when we compare them to appropriate controls."

The lack of biomarkers for these conditions makes it challenging to diagnose patients or recruit individuals into clinical studies. "This is why recruitment for these conditions is generally based only on clinical symptoms, resulting in highly heterogeneous cohorts," explains Alaedini.

One of the promises of immune profiling is that it can reveal immunologic phenotypes and biomarkers that would identify disease subsets with shared etiology and pathogenic mechanism. In addition, clinical treatment trials targeted at the identified disease subsets would have a greater chance of demonstrating efficacy, Alaedini points out.

"As part of this strategy," he continues, "we perform epitope mapping of immune responses to specific relevant antigens to achieve higher resolution or more detailed immune profiling."



Detailed epitope mapping of antigens during an evolving antibody response to an infection can help expose a pathogen's immune evasion mechanisms.

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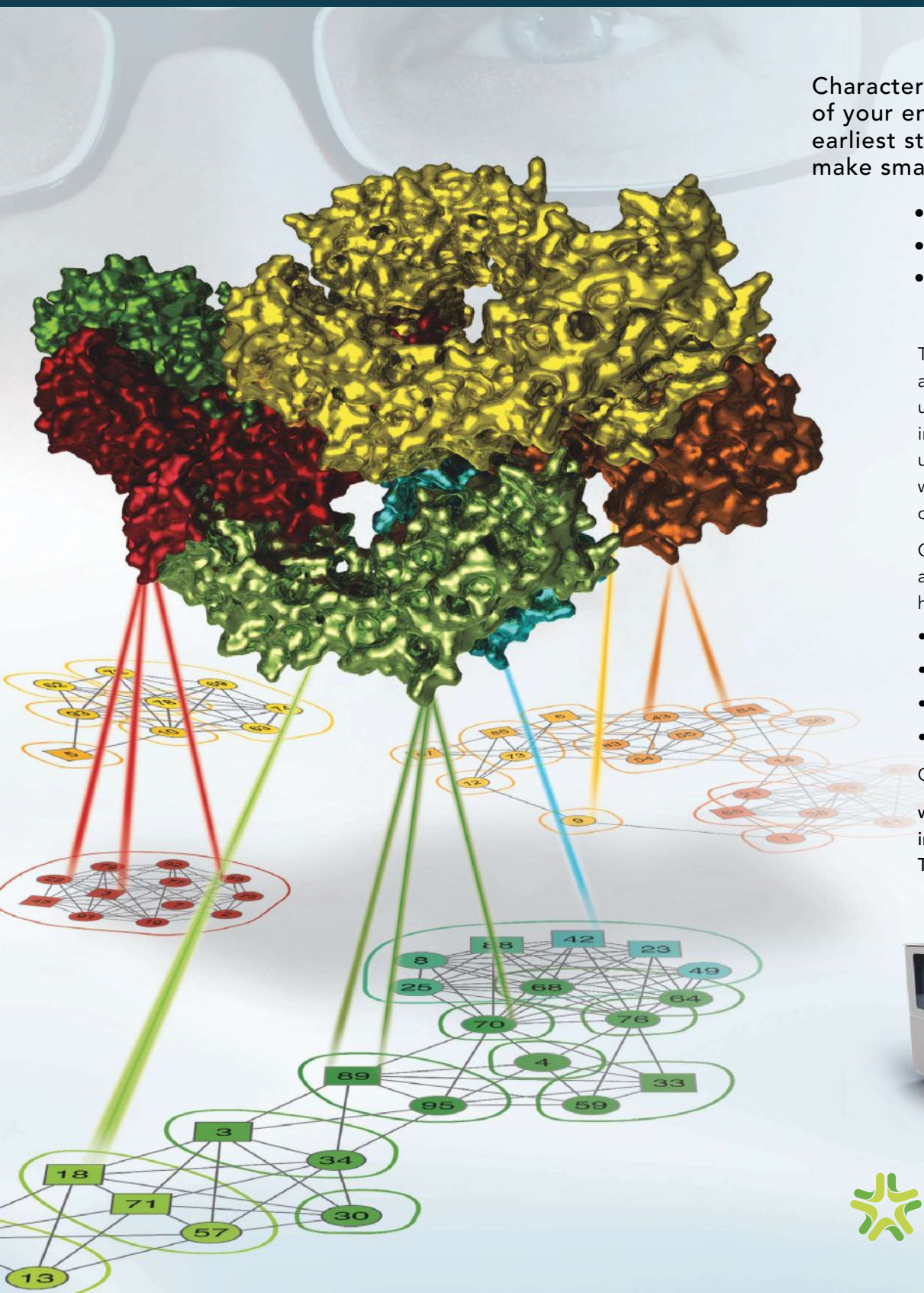
High Throughput mAb Characterization



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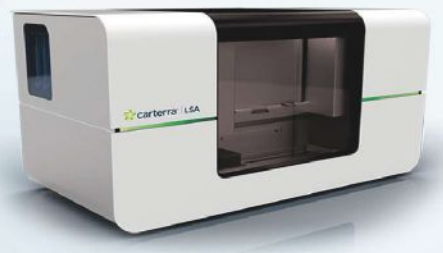

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In Lyme disease, after the early stage of the infection that is usually associated with skin lesions, the bacteria disseminate through blood and often cause additional manifestations, including early neurologic Lyme disease, such as neuropathy or facial palsy, sometimes followed months or even years later by more severe joint or late neurologic manifestations.

"At each of these stages of Lyme disease," Alaedini emphasizes, "the immune response is different." More antibodies are produced as the infection progresses, and they have increasingly higher affinities for their target antigens. What has not been undertaken, Alaedini adds, is a systematic attempt to understand how the various innate and adaptive immune responses evolve during these stages.

One of the working hypotheses in Alaedini's lab is that different antibody responses develop at different stages of the disease. To explore this hypothesis, the lab characterized the humoral immune response in patients who were in various stages of Lyme disease. The lab then consolidated its findings to trace the evolution of the humoral immune response.

The investigators found that an asynchronous antibody response develops against two major immunogenic regions of the VlsE protein. "VlsE," stressed Alaedini, "is the only known protein in the causative bacterium (*Borrelia burgdorferi*) that changes its sequence as the infection progresses."

Sequence modifications could help the bacterium evade the host immune system. While antibodies against one region of the protein developed early and their levels remained elevated, antibody responses against another region were found to be initiated only in later stages. "This finding gave us some clues into pathogenesis," Alaedini says. "It also helped us identify biomarkers associated with late-stage Lyme disease."

Knowledge of Lyme disease stages could help clinicians manage treatment. Most patients with Lyme disease respond well to antibiotics. However, some individuals develop persistent symptoms despite antibiotic treatment, even though an ongoing infection is no longer believed to be present. Alaedini's lab is trying to dissect and understand the causes of the immune dysfunction that appears to be involved and to identify biomarkers of PTLDS with the extensive use of epitope mapping.

Work in Alaedini's lab focuses primarily on linear epitopes, which are particularly helpful for diagnostics and are less challenging to incorporate into assays than conformational epitopes. "Conformational epitope mapping, especially in a high-throughput manner, will be more widely utilized in the next few years," predicts Alaedini. "It will supplement linear epitope mapping to help us improve our understanding of certain medical conditions and for disease phenotyping."

Epitope mapping could facilitate iterative vaccine design and reveal the diversity of responses to a vaccine.

In interrogations of conformational epitopes, a persistent challenge is the need for accurate predictions of protein folding and immunogenicity. "However, as we continue to have more advanced prediction algorithms and learn more about what makes an epitope in an immune response against an antigen, we will be able to generate more accurate predictions," declares Alaedini, "and we won't need to screen patient samples against large numbers of potential immunogenic sequences or domains."

This strategy would involve predicting the most significant epitopes of a protein. "We could then focus only on the protein regions of interest as potential epitopes without having to analyze the entire protein," concludes Alaedini.

Imaging antibody diversity

Many approaches to generate vaccines are converging on using protein subunits to elicit focused antibody responses. "But some of these protein surfaces are large," says Andrew Ward, PhD, professor at The Scripps Research Institute, "and antibodies have many potential ways to bind."

Epitope mapping has traditionally relied on introducing mutations and performing binding studies. However, many assays are indirect and nonspecific, or they may involve the surface immobilization of antigens, leading to an incomplete understanding of the humoral immune response.

These problems could be overcome by an alternative approach developed by Ward's group. "With our technique," Ward asserts, "we are imaging incredible antibody diversity in a very direct way."

Ward and colleagues are using an electron microscopy–based epitope mapping strategy to follow the evolution of neutralizing antibody development. In a study that applied this strategy to the immunization of rabbits with an HIV-1 envelope glycoprotein candidate, Ward and colleagues uncovered previously unidentified epitopes and revealed how antibody responses evolve. Essentially, the investigators generating a semiquantitative map of epitopes that are targeted in a polyclonal antibody response.

"This approach is performed in solution," Ward notes. "Therefore, it is not subject to some artifacts that occur with immobilization." It promises to capture the dynamics of the antibody development in real time, and to do so with unprecedented resolution. This approach, Ward hopes, will not only facilitate iterative vaccine design, but also enable the identification of signatures from infections, revealing the diversity of responses in humans to a vaccine.

For example, the approach could help explain why currently used vaccines elicit protective and durable immune responses in some people, even most people, but not all people. "Our imaging technique," Ward declares, "is part of a broader tool to understand very large complex heterogenous antibody responses." GEN

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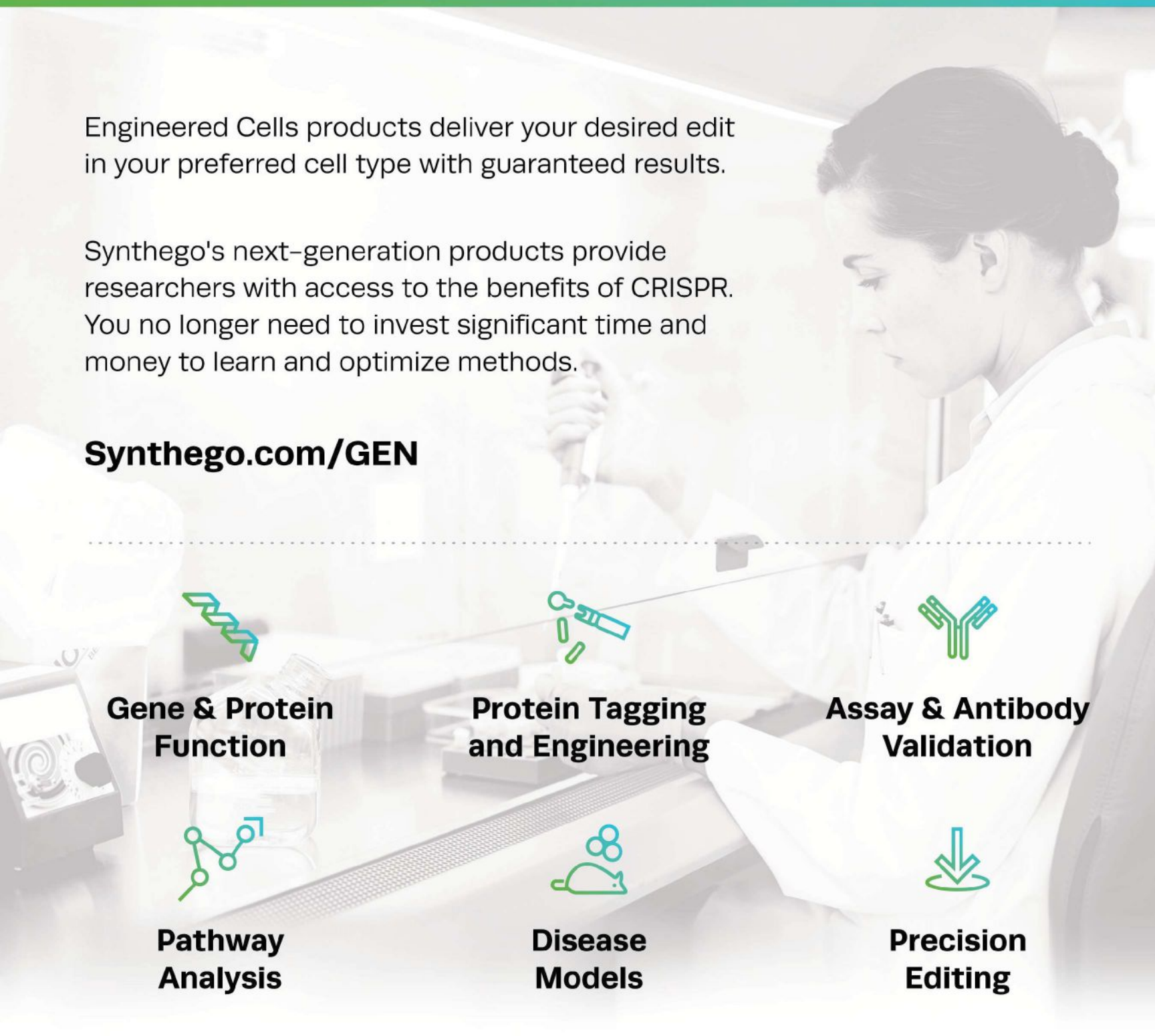

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Back to the Future:

Pre-CRISPR Systems Are Driving Therapies to the Clinic

By Emily Mullin

André Choulika, PhD

Paris-based Cellectis is a clinical-stage biopharma that uses gene editing and electroporation technology to create allogeneic immunotherapy products. When genetically editing a patient's own T cells, Cellectis uses the TALEN system rather than the CRISPR system.

A handful of biotechs are eschewing the CRISPR craze and making progress in the clinic by favoring older genome editing technologies

In 2011, **Cellectis** chairman and CEO André Choulika, PhD, told his employees that the Paris-based company was changing course. It was dropping the early genome editing platform that Choulika had been working on since the late 1980s, called meganucleases, and instead adopting a new technology—transcription activator-like effector nucleases, or TALENs.

It wasn't a hard decision for Choulika, even though he had pioneered the use of meganucleases—naturally occurring enzymes found in microbes and some plants—for genome editing. "You have to be technology-agnostic because technologies are usually meant to be obsolete after a time," Choulika says.

By mid-2012, a new gene editing technology emerged and sparked excitement among scientists, making modification of plant, animal, and even human genomes easier than ever before. That system, of course, is CRISPR-Cas9. As the hype for CRISPR built relentlessly over subsequent years, a small number of scientists, including Choulika, kept faith in older genome editing technologies: TALENs and ZFNs (zinc finger nucleases).

That perseverance is paying off.

Experimental therapies that use ZFNs and TALENs are advancing in the clinic, and TALEN-based crops are being harvested (*see sidebar "Farm Aid"*). The first gene edited products will be a reality soon, and they likely won't be made with CRISPR. In the near term, companies including Cellectis and **Sangamo Therapeutics** in California are committed to using the older platforms, especially as safety questions around CRISPR linger. But the fate of these older gene editing platforms remains to be seen.

Even as CRISPR has exploded, Choulika hasn't abandoned TALENs—though he did consider it. "We said, 'Wow, that looks super cool,'" Choulika recalls when asked about his team's first response to CRISPR.

Cellectis tried using CRISPR in early 2013, but after several months of experimentation, the company decided to return to the tried-and-tested TALENs, which it had been using to develop immunotherapies. "We found them to be more accurate, precise, and powerful, and we thought they would be safer for patients," Choulika says.

Cellectis was awarded the first European patent for using CRISPR in T cells in 2017, but the company has no intention of pursuing regulatory approval of a CRISPR-based therapy—at least not soon. Instead, the company is focused on getting its TALEN-edited chimeric antigen receptor (CAR) T-cell therapies approved in the United States and Europe.

Early days

In 1994, Maria Jasin, PhD, a developmental biologist at Memorial Sloan Kettering Cancer Center in New York, was the first researcher to induce a double-strand break at a specific point in mammalian chromosomal DNA using a restriction endonuclease. "Nothing like that had ever been done before," Jasin says. "It was such a dramatic result for me to see homologous recombination being induced so substantially." (In a review article published last year in *The CRISPR Journal*,

Maria Jasin, PhD

Sangamo scientists Jeffrey C. Miller, PhD (*left*), and Edward J. Rebar, PhD (*right*), hope that ZFNs hold the key to gene editing success in the clinic. The company's technology has been applied to varied conditions, including hemophilia A and hemophilia B, lysosomal storage disorders MPS I and MPS II, as well as beta thalassemia.

Fyodor Urnov, PhD, a pioneer of gene editing at Sangamo and now at the Altius Institute, hailed Jasin's breakthrough as "the first key discovery in the Age of Editing.")

Jasin's seminal work led to interest among scientists to use endonucleases to precisely snip DNA in the genomes of complex organisms. First came meganucleases, also known as homing endonucleases, which are still used by North Carolina-based **Precision Biosciences** but have been largely abandoned by academic researchers because of their limited range. (Founded in 2006, Precision recently won permission from the FDA to begin a clinical trial for a gene edited, allogenic CAR T-cell therapy for B-cell acute lymphoblastic leukemia and non-Hodgkin lymphoma. It's the company's first clinical-stage product.)

"That platform was really what started out the whole genome editing field," says Matthew Porteus, MD, PhD, a physician-scientist at Stanford University (and co-founder of **CRISPR Therapeutics**) who is developing gene editing therapies for children with genetic diseases. "But it's been the most challenging platform in terms of trying to reengineer a protein to recognize a new target site and maintain that high on-target activity and low off-target activity.

After meganucleases came ZFNs, a class of engineered DNA-binding proteins devised by Srinivasan Chandrasegaran, PhD, a professor at Johns Hopkins Bloomberg School of Public Health, and licensed by Sangamo. Between 1994 and 2001, scientists published several proof-of-concept papers for meganucleases and ZFNs.

ZFNs are composed of a cleavage domain and typically five or six individual zinc-finger motifs, each of which can recognize three base pairs of DNA. Sangamo has stayed the course with ZFNs, which it sees as having potential advantages over CRISPR technology.

"We've always had a great deal of confidence in the capabilities of [ZFNs] as a therapeutic platform," says Edward J. Rebar, PhD, chief technology officer at Sangamo. "In recent years, we've only gotten better at understanding them and getting better performance out of them."

Gene editing milestones

ZFNs entered the clinic in 2009—a major milestone for gene editing. In collaboration with a group led by Carl H. June, MD, at the University of Pennsylvania, scientists from Sangamo removed T cells from HIV patients, disrupted the CCR5 gene, and infused the gene edited cells back into their bodies. It was an attempt to stop the HIV virus from infecting new cells. A few participants in the trial have been able to stay off HIV drugs since receiving the experimental treatment, but most have not.

Cellectis developed the next gene edited ex vivo therapy, a donor-derived T-cell therapy edited with TALENs, and licensed it to pharma companies **Servier** and **Pfizer**. Called UCART19, the therapy entered clinical trials in 2016, administered to two infants with aggressive B-cell acute lymphoblastic leukemia. In January 2017, researchers reported that the therapy had led to remission in both cases. But the end of 2017 brought tragic news: a patient died after receiving UCART123, another allogenic gene edited therapy developed by Cellectis.

For Sangamo, 2017 was also a landmark year. The company became the first to attempt in vivo gene editing in a patient. That November, a 44-year-old man named Brian Madeux was the first individual to receive Sangamo's SB-913 for Hunter syndrome, or

Farm Aid

The first gene edited food product to hit the market will be one made with the TALEN system, not the CRISPR system.

Calyxt, whose parent company is **Cellectis**, plans to introduce its gene edited soybean oil to the United States this year. Calyxt was founded in 2010 after Choulika approached Dan Voytas, PhD, a professor of genetics and cell biology at the University of Minnesota, who was developing the first TALENs alongside Adam Bogdanove, PhD, at Iowa State University.

Until recently, soybean oil was often chemically treated through a process called hydrogenation to make it more amenable for use in cooking and to extend its shelf life. But this process gives rise to trans fatty acids, which the FDA banned in June 2018 because of a link to "bad" cholesterol and heart disease.

This negative association means consumers have gravitated toward other, healthier oils. But soybean oil is cheap to produce, and the United States is the top soybean exporter and producer in the world. In 2017, U.S. farmers grew nearly 90 million acres of the legume.

Calyxt wants to make soybean oil an attractive option for both farmers and consumers. It is making a "heart healthy" version that resembles olive oil or canola oil, using TALENs to inactivate a pair of genes in the soybean plant to change the fatty acid composition.

In fall 2017, Calyxt harvested 17,000 acres of the TALEN-edited soybean and then crushed the beans to make the oil. Voytas expects to begin selling the gene edited oil in early 2019.

"Right now, we don't see any limitations to TALENs," Voytas says. "We can make the types of gene edits we want to make in our crop varieties. But of course, technology advances quickly, and we're going to have to make sure that we adapt TALENs to gene editing approaches that are going to give us products of value." Next, Calyxt plans to roll out a high-fiber wheat edited with TALENs. The company completed a field trial in 2018 and is conducting food testing on the grain it grew. ■

mucopolysaccharidosis type II, a rare hereditary lysosomal storage disease. The ZFN-based genome editing approach is designed to insert a corrective gene into the patient's liver cells.

"One of the key things about our in vivo approach is that we're using very similar zinc fingers across the board, so success in one will mean success in the others," says Edward Conner, MD, Sangamo's CMO.

Edward Conner, MD

Today's landscape

With CRISPR all but established as the gene editing tool of choice in academia, researchers think the future of TALENs and ZFNs will likely be limited to the companies using them to commercialize products. There are already hundreds of labs and several companies using the CRISPR platform. "What's rapidly going to happen is that the number of clinical trials using CRISPR is going to zoom past TALENs and ZFNs. It's going to be exponential," Porteus predicts.

Jasin thinks TALENs and ZFNs might find a role as alternatives to CRISPR if off-target effects end up being a concern for certain indications. Older gene editing technologies "could always be on the back burner for more specialized use," she says. "If for a particular disease there's a really well-developed zinc finger that's not known to have off-targets, and CRISPR does, then there would be a rationale for using that."

Porteus, whose lab raised an alarm last year about the existence of preexisting immunity in humans to Cas9 proteins, agrees. He says researchers might look to use TALENs and ZFNs again if CRISPR use in the clinic raises safety concerns. This may be less of a problem with TALENs, which originate from a type of bacteria that infects plants, and ZFNs, which are artificial enzymes to which humans haven't been exposed.

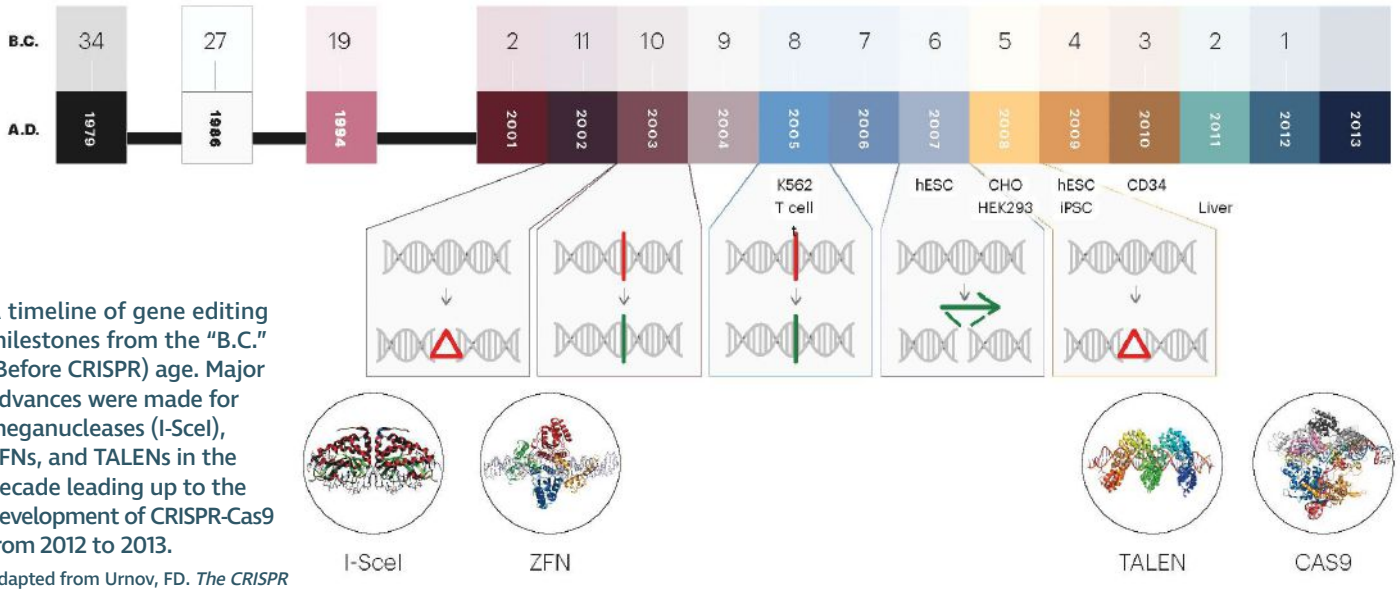
"What if there is an unexpected event with CRISPR technology? Depending on what that adverse event is, that might rejuvenate interest in the older technologies. So I'm glad they're there," Porteus says.

Sangamo's Conner sees benefits in the use of different gene editing techniques "because they have the potential to address a lot of different diseases."

Phase II trials of UCART19 will begin later this year. Cellectis' Choulika predicts that three of the company's TALEN-edited cancer therapies will be on the market by 2022. Meanwhile, Sangamo is enrolling children as young as 5 years old in its Hunter syndrome trial—a testament to the therapy's perceived safety. And last December, Sangamo announced the beginning of a Phase I/II trial for hemophilia B using a ZFN approach called SB-FIX to introduce the factor IX clotting factor gene into liver cells.

For now, TALENs and ZFNs have the early lead in the gene editing clinical race, but as several CRISPR therapies enter trials, the fate of these older gene editing platforms is uncertain. "It remains to be seen if there's any difference between a ZFN and a TALEN and a CRISPR once you enter the clinical arena," Porteus says. GEN

Emily Mullin (emily.mullin@gmail.com) is a freelance contributing writer based in Baltimore, MD.



A timeline of gene editing milestones from the "B.C." (Before CRISPR) age. Major advances were made for meganucleases (I-SceI), ZFNs, and TALENs in the decade leading up to the development of CRISPR-Cas9 from 2012 to 2013.

Adapted from Urnov, FD. *The CRISPR Journal* 1: 34–46 (2018).

Antibody Discovery Looks Over the Horizon

Abcam platforms can probe immune interactions and keep biologics on target

Joyce Young, PhD, VP of Custom Solutions

Jamie Campbell, PhD, Head of Custom Solutions UK

Valeria Busygina, PhD, Head of Custom Solutions US

Antibody discovery platforms have come a long way since the initial steps toward understanding what antibodies do and how to harness them.

GEN recently spoke with Joyce Young, PhD, VP of Custom Solutions, Jamie Campbell, PhD, Head of Custom Solutions UK, and Valeria Busygina, PhD, Head of Custom Solutions US, three experts in antibody development platforms from Abcam.

GEN: Why are antibody discovery platforms increasingly important and in demand?

Dr. Campbell: In oncology, antibodies are being viewed as the mainstay of therapy. That means people are moving away from the other types of therapies, such as small molecules, which are relatively inexpensive but not as specific as antibodies.

Dr. Young: The therapies coming out of antibody discovery are transformational: There are now so many different targets and combinations of those targets, to go after. Antibodies are moving into specific patient stratification to do that.

Bispecifics, which are big at the moment, join different specificities of antibodies together. The industry is even using antibodies to target and reprogram T cells. Antibodies continue to be the biggest growing drug market, and you have oncology therapies coming off the back of the immunoregulatory space where antibodies, instead of targeting the tumor, are targeting misregulation of the immune system.

GEN: This focus on antibodies as therapies means the tools to study them are advancing. How is this changing the way science uses antibodies?

Dr. Campbell: Originally, researchers would screen large compound libraries. Now, they're doing antibody discovery, which is more efficient. Antibodies and antibody libraries are designed and targeted so rather than fishing in a large pool and seeing what sticks; you're stacking the cards in your favor and biasing that pool.

GEN: The phage display technique—inserting a gene into a bacteriophage so that a protein of interest may be expressed on the bacteriophage's surface—was developed back in the 1980s. For decades, the technique remained difficult and expensive, but it has started to become more accessible. What accounts for the change?

Dr. Busygina: New platforms such as our AxioMx platform have sped this process up. We can now develop antibodies with the benefit of binder enrichment from diverse libraries within days to weeks rather than months or years.

Phage display allows us to target those molecules that are difficult to zero in on with other methods, that is targets that are toxic or not immunogenic. It also allows for more intelligent design of antibodies by predicting the sequence that would bind to a certain molecule, and by modifying the antibodies in vitro to procure the properties we want. Unlike immunization approaches, we control the input and outcome of every phage display–based antibody discovery. It is also much quicker, so we can achieve our goals faster.

AxioMx is a high-throughput phage display antibody discovery platform, using high-diversity libraries of antibody single-chain variable fragments (scFvs) fused to the P3 coat protein of an M13 bacteriophage. Our libraries are designed to allow rapid cloning to give a full IgG recombinant antibody, all created using animal-free technology. This type of antibody discovery is fast and cost-effective, and it has benefits across a wide array of applications.

GEN: Another approach to antibody discovery is next-generation sequencing (NGS). The method enables the discovery of many more unique clones than traditional approaches such as hybridoma generation, resulting in a pool of rationally selected antibodies. This technique gives researchers access to a comprehensive database of antigen-specific binders, helping to increase the chances of identifying the best antibody for the task at hand. Tell us about your NGS Platform?

Dr. Young: It took us six to eight years to develop our NGS platform. Much of that was around getting the bioinformatics together and applying NGS to the rabbit immune response. The rabbit immune system brings many advantages, with diversity generated by gene conversion in addition to somatic hypermutation. It was rather a complex beast to tackle with NGS, but it's now a robust platform that can go out to customers.

Dr. Busygina: The NGS approach allows us to look at the whole immune response in depth and breadth. It reveals what the whole immune response looks like, not just a few clones. Then, we use a bioinformatics approach to select a set of binders that will be the most beneficial to our application.

GEN: Abcam has long been known as a specialist producer of antibodies. How else do you work to meet your clients' needs?

Dr. Young: Now we're able to support our customers from bench to bedside. We're able to take clients from wondering if what they have is a target or not, to being supplied with reagents that are produced under regulated manufacturing processes and that can be used to support diagnostics and patient stratification.

Working with clients is key. Abcam develops some of these therapeutics, and we help define which patients would benefit from them. We also bring the reagents that can allow us to do patient stratification and to progress these drugs through clinical trials and pharmacokinetic analysis.

Abcam provides support from beginning to end, validating the target, deriving the antibody against it, deriving support reagents, and supporting antibody drugs through development.

GEN: Artificial intelligence (AI) is advancing all areas of science and technology, and one of these areas is antibody discovery. How critical will AI be in the future of antibody discovery?

Dr. Campbell: Computational tools and bioinformatics are advancing at a rapid rate, not just in science but in all technology. I see these tools, in the form of AI being applied to the big data sets we now have from things like NGS. A lot of the initial analysis from an NGS library will be done using these AI tools, and that will essentially be the screening component. I can only see the NGS approach improving as our tools get better at predicting and understanding which clones we should make and select first. This means we'll be able to screen less, give antibodies to the customer quicker, and provide them with the right tool from the start.

Phage display allows us to target those molecules that are difficult to zero in on with other methods, such as targets that are toxic or not immunogenic.

Dr. Busygina: I am working directly with these tools in the laboratory, and I believe AI will ensure the discovery of new antibody platforms. AI will allow us to do things faster, but it will also change how things are done. Initially, DNA sequencing was a tremendous effort, but now it is cheap and easy and fast. We want to move the antibody field to a similar paradigm, where we can achieve greater success in developing specific properties in each antibody we develop.

We can now generate a lot of data regarding the immune response to a particular antigen, and regarding the evolution of antibodies in vitro. I can imagine that in the future we'll combine those two approaches to allow in silico antibody prediction. We'll be able to predict the properties of certain antibodies or predict the sequences we'll need to put in the complementarity-determining region to develop antibodies bioinformatically.

GEN: Where will the next fundamental shifts in antibody discovery come from?

Dr. Young: From combining approaches and working out where the center of the Venn diagram of knowledge and technical ability sits. Going forward, I see us being able to really harness our knowledge of the immune system. Phage display is the way the AxioMx platform has harnessed the affinity maturation stage, a clever molecular biology tool to give rapid affinity maturation—more rapid than any other phage display out there. That has meant applying knowledge of molecular biology to technology in a clever way.

There's no reason we can't continue to do that. Synergy between different technologies is often where you get the "aha" moment and advance things, so we're lucky to have three tremendous platforms: NGS, phage display, and RabMAb®. ■

Filtration Focuses on Capacity and Concentration Pain Points

By Gareth John Macdonald

Besides staying ahead of mAbs, the filtration sector is catching up with gene and cell therapies

A therapeutic protein or monoclonal antibody (mAb) can be turned into an effective therapy and a revenue-generating product only if it can be efficiently filtered from the process stream. If a product is inefficiently filtered, the overall process slows, just as surely as it may slow due to upstream bottlenecks. Yet upstream limitations, not filtration problems, sometimes seem more amenable to technological fixes, if not more urgent. For example, upstream limitations have prompted improvements in bioreactor technologies that have allowed manufacturers to grow cell cultures at higher densities.

In recent years, however, efficiency-enhancing development has become less upstream-centric. Drug manufacturers have started to recognize that if they are to benefit fully from upstream improvements, they need to improve downstream operations, too.

In general, drug manufacturers are looking for increased filtration capacity. Besides working to meet this demand, filtration technology developers helping biomanufacturers handle higher product concentrations, reduce wastage of costly inputs and valuable outputs, simplify workflows, and overcome the special processing challenges posed by gene therapies and cell-based therapies.

Raising concentrations

The drug industry's focus on higher dosage biopharmaceuticals is influencing filtration technology design. "Concurrent with the trend toward the development of drug product for subcutaneous delivery, we are seeing requests for sterilizing-grade filters that perform reliably with concentrated drug product and drug substance," observes Oliver Triebsch, senior director, marketing, **Pall Biotech**. He adds that sterilizing-grade filter membranes which can sustain stable flow rates are particularly sought after.

Demand for enhanced filtration membranes has also been recognized by **GE Healthcare Life Sciences**. "We have started seeing more interest and potential in modified membranes to fit its purpose better, such as charged depth filters for clarification, but also new applications where a membrane technology is used in new situations," comments Fredrik Lundström, the company's product manager.

"One of the most interesting areas," he continues, "is using membrane structures for chromatography in bind-elute mode." In chromatography, flow-through mode is when the column is being flushed with solvent. In contrast, bind-elute mode is when elements in the process stream are being separated by binding to the column.

"Flow-through membrane chromatography has been around for some time, but the novel fiber-based technology, FibroSelect, significantly increases productivity," Lundström asserts. "Other advantages of the FibroSelect technology are possibilities to closed processing and completely disposable devices."

Christine Gebski, vice president of product management and field applications at **Repligen**, has also noted the drive toward higher concentration products. "Developers of mAbs," she says, "are striving to formulate bulk drug substances to higher protein concentrations, with concentration targets rising from less than 100 mg/mL to now as high as to 200 mg/mL."

Gebski notes that the trend has been factored into Repligen's technology development efforts. "Protein formulation is achieved with tangential flow filtration (TFF) using flat-sheet cassettes in classic ultrafiltration and or diafiltration operations," she details. "With TangenX™ SIUS™ and Pro TFF, we have demonstrated the ability to achieve these higher concentrations, with either single-use or reusable product options."

Reducing costs

Cost efficiency is another driver, one that is becoming more important as biopharmaceutical active ingredients become more valuable. Also, these ingredients are being used to create increasingly valuable products.

Cost efficiency is another driver, one that is becoming more important as active ingredients become more valuable.

"Subcutaneous treatments, which require high-concentration formulations, pose extra challenges in terms of concentration and buffer exchange steps, as well as in subsequent fluid handling, sterile filtration, and filling steps," Triebsch elaborates. "These challenges include extensive processing times, yield loss in holdup, and additional cumulative sheer damage to the molecules."

"Higher concentration means higher value of product per unit volume," he adds. "End users are particularly interested in devices that can allow them maximize the recovery of these feeds post-filtration, particularly during process development when fluid is scarce."

Increasing cost pressure is changing bioprocessing in other ways, according to Lundström. "Connecting process steps to avoid downtime is becoming more relevant,"

At left. To achieve high efficiency, a filtration workflow may incorporate single-use technology or enhance process automation—or it may do both, suggests GE Healthcare Life Sciences, which developed the ÄKTA readyflux, an automated single-use filtration system for pilot and small-scale manufacturing. The system is intended for crossflow (also called tangential flow) filtration applications in both upstream and downstream workflows.

he tells *GEN*. "For filtration, users have started to use approaches such as single-pass TFF to quickly concentrate the product a few times."

He points out that GE Healthcare is responding to a trend toward more concentrated protein solutions, where holdup volumes are becoming critical. "The recently launched GE Healthcare ÄKTA™ readyflux," he asserts, "provides high-quality holdup volume at the capacity provided."

Eliminating bottlenecks

Balancing downstream filtration capacity with higher upstream productivity is another very important innovation driver in the filtration sector, says Tony Hunt, president and CEO, Repligen. "Biopharma companies and contract manufacturers want flexible filtration solutions that improve yield, simplify workflows, eliminate other unit operations, and streamline the manufacturing process," he tells *GEN*.

Repligen entered the filtration technology market in 2014 with the acquisition of the ATF platform from **Refine Technology**. In 2017, Repligen further expanded its offering by buying Spectrum, which brought Repligen membrane separation and hollow fiber filtration technology.

While incorporating new technologies, Repligen has maintained its position as a major supplier of Protein A, which is still the most widely used material in chromatography resin production. By spanning that which is enduring and that which is emergent, the company, Hunt suggests, has gained unique insight into the filtration sector.

"Repligen has really diversified and expanded over the past five years," he declares. "Today, Protein A ligands represent less than 20% of the revenue of the company. We have transformed from being an OEM provider to being a direct-to-customer bioprocess provider.

"We have innovated and brought high-impact products to market like XCell ATF perfusion systems, OPUS prepacked chromatography columns, and both hollow-fiber and flat-sheet TFF systems. Also, we are addressing major pain points in the biologics manufacturing workflow. Consequently, we are now considered one of the leaders in the field of filtration and chromatography."

Upstream bioprocessing may be simplified and intensified, Repligen asserts, if the bioreactor is attached to a cell retention device. For example, Repligen says that its ATF System is capable of 100% cell retention and 80% faster implementation in perfusion operations. Available in single-use or stainless-steel formats, the ATF is based on alternating tangential flow technology, that is, the action of a diaphragm moving upward and downward within a pump head.

It's a mAb, mAb, mAb, mAb world

At the molecular level, modern mAb-based drugs and therapeutic proteins differ significantly from older products, both in terms of how they are designed and how they interact with therapeutic targets. However, from a filtration standpoint, little has changed in how these molecules are recovered from the process stream, notes Triebsch.

He allows that current mAbs are very similar to the older mAbs, both in terms of concentration and molecular behavior. Nonetheless, he insists that "some newly engineered mAb variants—such as bispecific antibodies (bsAbs), antibody fragments, pro-antibodies, fusion proteins, to name a few—can pose additional filtration challenges."

The drug industry already knows how to filter mAbs, says Lundström, who tells *GEN* that, for the most part, current technologies are sufficient: "The mAb process has been more or less a platform for many years, and there has been a true revolution in terms of titer development due to better cell line development and media optimization. Traditional therapeutic proteins in CHO cells do not come close in terms of mass of product produced per liter."

"Filtration technology has not changed as such," he continues, "but we see some challenges in harvest in single-use operations, and ion-exchange membranes are sometimes seen in some early clinical trials."

Gebski also says the evolution of biopharmaceuticals has had less of an impact on the development of filtration technol-

ogies than other factors: "Irrespective of the scientific evolution in antibody development, filtration technologies have been fairly conserved."

"For instance, with TFF operations, the choice of filter molecular weight cut-off to be used is driven by the size of the molecule, which remains the same for a given type of therapeutic protein," she details. "Also, some advances in the flat-sheet cassette screen construction have enabled high-protein concentrations to be attained."

Future demand

The drug industry's interest in cell and gene therapies is often cited as a driver of processing technology innovation, including innovation in the filtration systems sector. "In the gene and cell therapy markets adjacent to the classic mAb, recombinant protein space," Triebsch tells *GEN*, "there will be demand linked to the sterile filtration of viruses and other gene-delivery vehicles such as liposomes and exosomes."

Triebsch predicts that the when the technology sector develops filtration products, it will consider biopharma's interest in single-use, disposable systems: "We will see an ongoing trend toward more presterilized devices, which embody ready-to-process functionality and are integratable into single-use assemblies. We will also see improvements in devices such as transfer tubing, especially for high-concentration formulations."

The growing influence of the cell and gene therapy sectors is also being factored into GE Healthcare's development of all processing technologies, including its filtration systems, says Lundström. "The number of cell and gene therapy indications has increased tremendously the last couple of years," he tells *GEN*, adding that this is a particularly attractive opportunity for filtration technology developers.

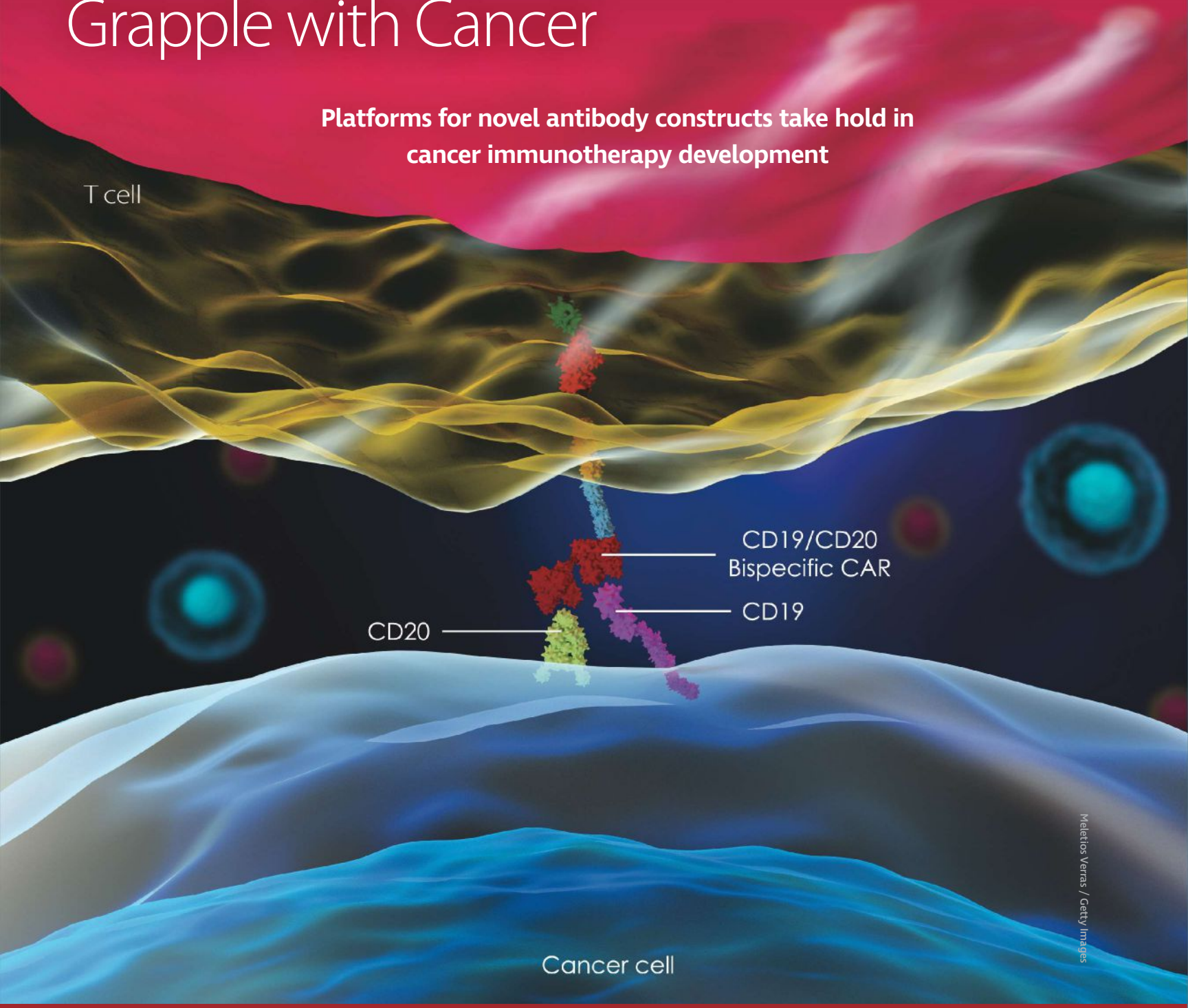
"Typically, the production of viral vectors is not as standardized as that of mAbs, and it includes more filtration and fewer chromatography steps," he continues. "However, it is more important to have closed processing. In multiproduct production, utilizing disposable technology is a necessity."

To cater for this demand, GE Healthcare has developed a range of hollow-fiber technologies that enable closed and sterile operations during TFF. "Our novel FibroSelect technology is anticipated to be a great leap forward in terms of chromatography operations," asserts Lundström. "All wetted parts are disposable and support completely closed assembly." GEN

GEN TRANSLATIONAL MEDICINE

Bispecific, Multispecific Antibodies Grapple with Cancer

Platforms for novel antibody constructs take hold in cancer immunotherapy development



Meletios Verras / Getty Images

By Ian Clift, PhD

Cancer immunotherapy has been advancing on several fronts, most strikingly in the direction of checkpoint inhibition and chimeric antigen receptor (CAR) T-cell therapy. Another front, however, is about to see its share of action. Here, newly engineered bispecific and multispecific antibodies will be put to the test. Such antibodies may engage two or more antigens at once, serving as force multipliers that can exploit opportunities beyond the reach of monospecific antibodies, whether they are deployed solo or in teams.

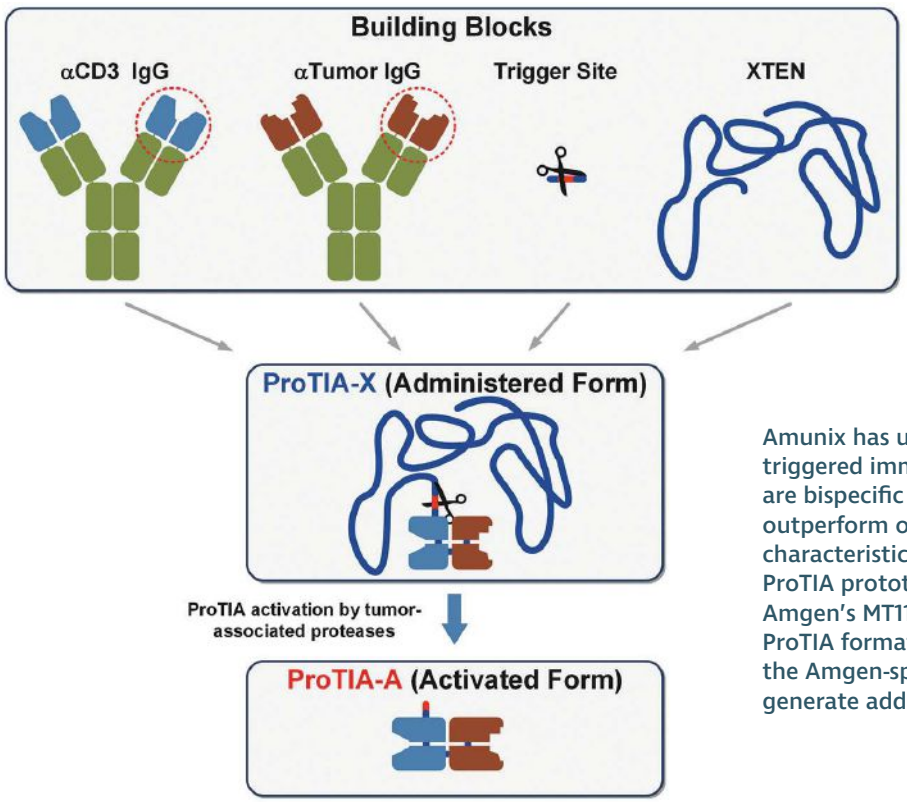
Although monospecific antibodies are beginning to show their limitations, they should be recognized as part of a sequence of antibody-based cancer immunotherapy developments, a sequence that reaches back at least as far as the Nobel Prize–winning efforts of James P. Allison, PhD, and Tasuku Honjo, MD, PhD. Allison's work on the CTLA-4 led to the first FDA-approved checkpoint inhibitor drug, ipilimumab (Yervoy, **Bristol-Myers Squibb**), whereas Honjo's discovery of PD-1 led to the development of anti-PD-1 drugs such as pembrolizumab (Keytruda, **Merck**). These drugs and other checkpoint inhibitors have profoundly impacted the treatment of cancer.

An alternative cancer immunotherapy approach, namely CAR T-cell therapy, has also demonstrated its potential to combat cancer. In this approach, T cells are engineered to launch sustained attacks on tumors. Although CAR T-cell therapies clearly have fight in them, they may cede some anticancer glory to bispecific antibodies (bsAbs). The first FDA-approved bsAb to directly compete with CAR-T was the CD19/CD3 drug blinatumomab (Blincyto, **Amgen**). It was introduced in 2014 for indications in B-cell precursor acute lymphoblastic leukemia.

Even while monospecific antibody–based checkpoint inhibition therapies and CAR T-cell therapies continue to be improved, bispecific and multispecific antibodies are shaping up as a cancer immunotherapy options that may provide significant advantages. At present, companies such as **Amunix Operating**, **Invenra**, **Glycotope**, and **Xencor** are working independently and in collaboration with larger pharmaceutical companies, such as **Novartis**, **Daiichi Sankyo**, and **Roche**, to bring bispecific and higher-order antibodies into the cancer immunotherapy market. Fundamentally, their engineered expression platforms focus on streamlining novel antibody development, reducing the risk factors to patients, and optimizing tumor destruction.

Increasing Selectivity

bsAbs emerged with the technologies developed by two pioneering companies Amgen and **MacroGenics**. Amgen introduced the BiTE platform; MacroGenics, the DART platform. Despite the availability of such platforms, it can still be a challenge to



XTEN is a Key Component of Pro TIA
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- Provides long circulation half-life
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- Minimizes aggregation risk
- Facilitates manufacturing

Amunix has used its XTEN platform to develop protease triggered immune activator (ProTIA) molecules. These are bispecific T-cell activators that are designed to outperform other bispecific formats with respect to characteristics such as half-life and safety. An early ProTIA prototype molecule, one that incorporated Amgen's MT110, allowed Amunix to demonstrate the ProTIA format's advantages. Amunix is now replacing the Amgen-specific portions of the prototype to generate additional ProTIA molecules.

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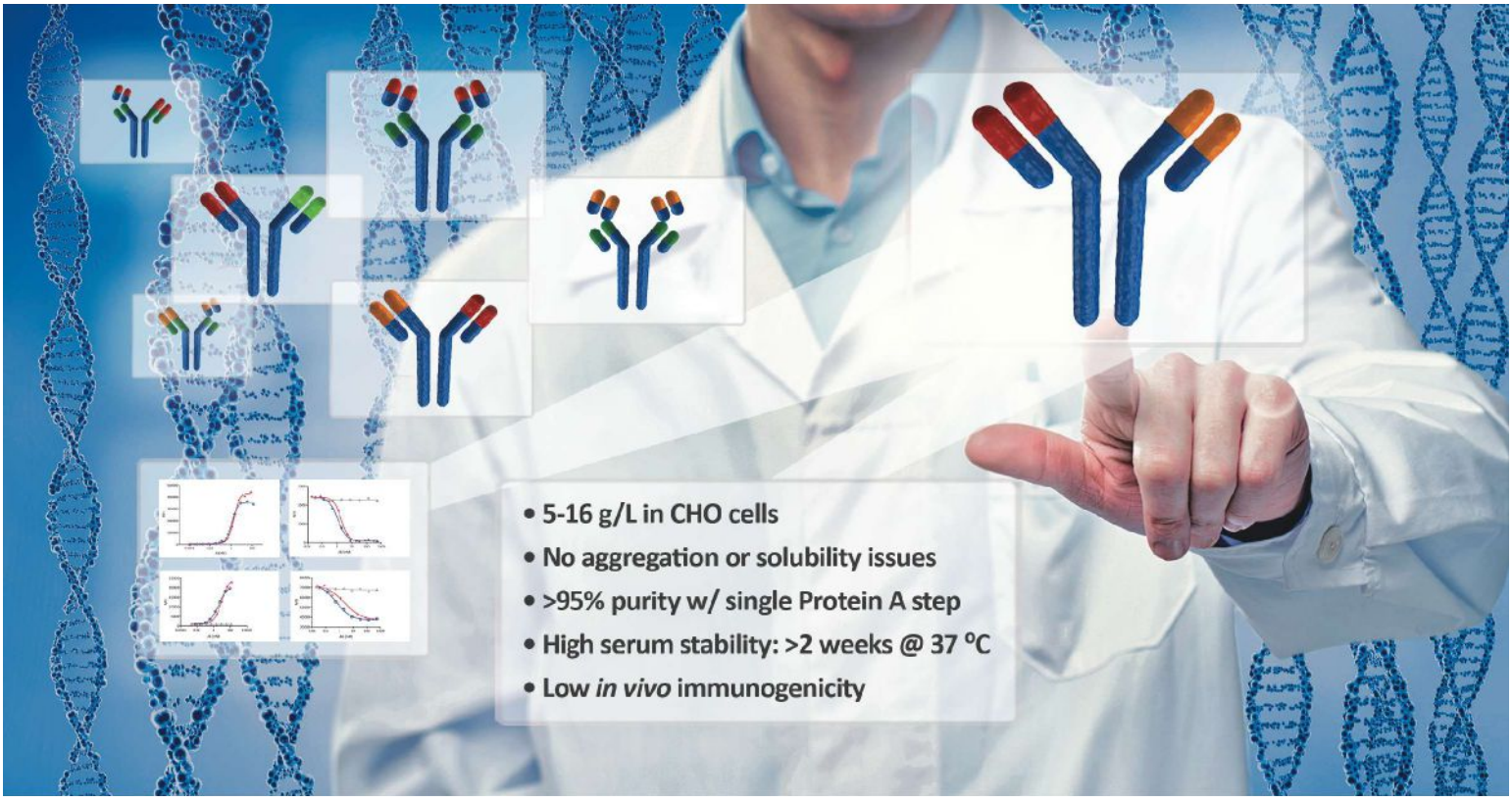

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produce bsAbs that incorporate an Fc domain, suggests John Desjarlais, PhD, senior vice president of research and CSO at Xencor. "If you don't have an Fc domain," he says, "you have a very short half-life," necessitating low and frequent injections or continuous infusion in patients.

Xencor's solution was to build a robust and GMP-scalable bispecific platform that includes an engineered Fc domain for the antibody, ensuring that antibodies produced with this platform would have a longer half-life in vivo. Xencor's XmAb Fc platform increases this efficiency of heterodimer Fc formation to 95% out of the gate.

"If I want to make a heterodimeric Fc domain, one that is different on either side," he says of a traditional process, "I'm going to get a mixture of 50% of the heterodimer, and 25% of the different homodimers by comparison."

To improve efficiency yet further, Xencor has engineered an additional feature in the Fc domain. "We perturb the isoelectric point on either side of the Fc heterodimer through substitutions in the Ch3 domains," Desjarlais details. "The idea behind that was, we would have an ability to very easily separate out the small amount of contaminating homodimers just by using ion exchange chromatography."

Xencor is exploring bsAbs that act as dual checkpoint inhibitors, such as anti-PD-1/CTLA-4 and CTLA-4/LAG-3. The field has learned that cancer evolves to suppress the immune system by engaging different pathways meant to protect the body against autoimmunity.

Single checkpoint blockers on the market such as nivolumab (Opdivo; anti-PD1) and ipilimumab (Yervoy; anti-CTLA-4) have been used in combination to improve anti-tumor activity, but this approach, says Desjarlais, comes at the cost of increased toxicity. Dual-targeting antibodies may promote less toxicity by more selectively targeting the tumor reactive T cells. "The idea is to turn off the brakes," he explains, "and the more brakes you can hit at the same time, the more you can activate those tumor T cells."

In addition to checkpoint inhibitors, Xencor has been successful in establishing two Phase I trials in collaboration with Novartis involving T-cell-engaging bsAbs; one that has an AML indication and binds to CD123 on AML blasts and CD3 on T cells, and a second that binds to CD20 on malignant B cells and CD3 on T cells. The company has a third wholly owned bsAb that binds CD3/SSTR2 (somatostatin receptor 2). Currently in Phase I trials, this bsAb is being explored with dose escalation in neuroendocrine tumors.

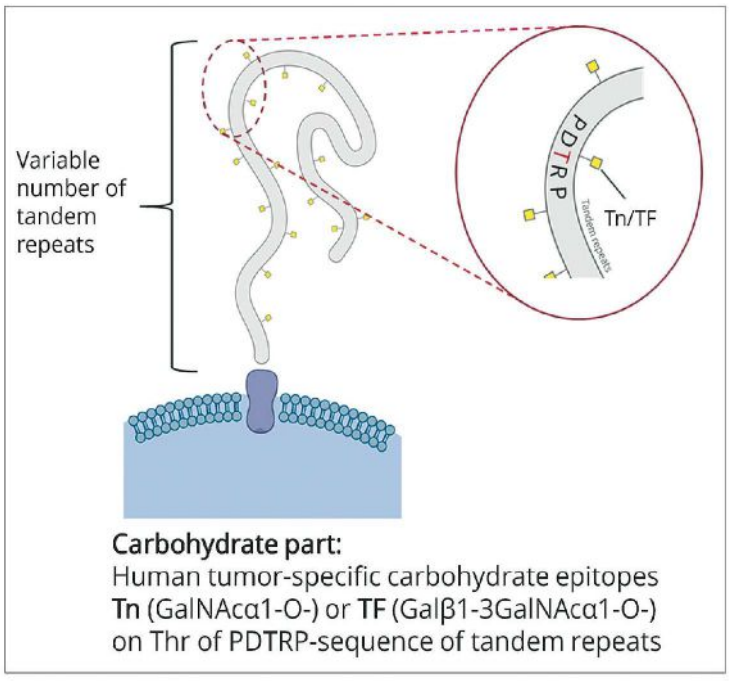
"CD3 bispecifics would be considered direct competitors to CAR-T," asserts Desjarlais. CAR T-cell therapies require weeks of preparation including cellular extraction from a patient, engineering in vitro, culturing, speculative dosing, and continued growth in vivo. In contrast, Desjarlais points out, "a bispecific is something in a vial that you have in the pharmacy."

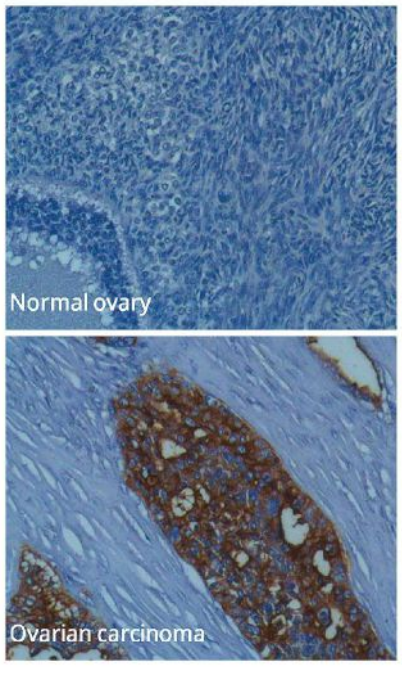
"With a bispecific," he emphasizes, "you know exactly what you're putting in."

T-cell engagers

Volker Schellenberger, PhD, president and CEO of Amunix, affirms that the challenge of the CAR T-cell therapies is that they must be individually created for each patient. "Another challenge," he says, "is that you are injecting live cells into a patient. So, it is very difficult to control what happens to them. They can even multiply in that person."

"We need to somehow mitigate the toxicity of these T-cell engagers," insists





Aberrant glycosylation patterns specific to cancer cells can be targeted by engineered antibodies, such as those developed by Glycotope using its GlycoExpress (GEX) platform. For example, the company's PankoMab-GEX antibody recognizes a tumor-specific epitope of MUC1 (TA-MUC1). *Left*: Schematic illustration of MUC1 highlighting the PDTRP motif, which has a conformational epitope induced by the carbohydrate antigens Tn or TF. *Upper right:* Normal ovarian tissue, which lacks PankoMab-GEX staining. *Lower right:* Ovarian carcinoma detected with PankoMab-GEX, which can recruit the immune system to destroy tumor cells.

Schellenberger. "If you have a protein-based drug, then you could give it right away, instead of after the several weeks it takes to develop an individualized CAR T-cell therapy; that would be a big benefit to the patient."

Amunix has developed a new format of bispecific T-cell engagers that can be delivered in a low dose with lower toxicity using XTEN technology, an alternative to PEGylation. "The T-cell engager," Schellenberger explains, "works like an adaptor molecule. It bridges the tumor and the T cell." XTEN is a protein polymer that is engineered to behave like polyethylene glycol (PEG) which is attached to bsAbs to increase their half-life in vivo without the need for an Fc domain.

"XTEN has evolved into kind of a Lego kit for pharmaceuticals," Schellenberger notes. "It allows us to make very complex molecules which by other means we just couldn't produce."

The company's lead XTENylated bsAb, AMX-268, is in preclinical development. It is a T-cell engager that binds to CD3, a T-cell receptor (TCR), and EpCAM, an adhesion molecule overexpressed in 80% of solid tumors.

"We give the drug in an inactive form and convert it to the active form only when it is in the tumor environment," Schellenberger says. The company's pro-drug is activated by the inflammatory process found primarily within the tumor microenvironment, reducing off-target toxicity and increasing antitumor selectivity, "so that if our molecule finds that target in a healthy organ, it will still leave it alone."

The active form of the drug is smaller than typical Fc-containing intact antibodies, allowing it to be removed easily and rapidly through the kidney. Schellenberger's data suggests that AMX-268 may have lower immunogenicity and a lower toxicity profile among other potential EpCAM-targeting T-cell engagers such as Removab (**Fresenius Biotech**) and the investigational MT110 (Amgen).

Moving from mono- to bispecific antibodies

One company that is leveraging its success in developing monospecific antibodies into bi- and trispecific antibodies is Glycotope. According to Anika Jäkel, PhD, the company's director of preclinical pharmacology and cancer immunology, "Glycotope has strong expertise in glycobiology and focuses on the generation of antibodies against tumor-specific glycoepitopes."

The company's first-in-class mAb, Gatipotuzumab, targets the tumor-specific epitope TA-MUC1, a novel combined carbohydrate/peptide conformational epitope on the tumor marker MUC1 (mucin-1). This antibody shows broad therapeutic potential in 80–100% of its main solid tumor indicators (that is, ovarian, lung, and breast cancers).

"Our most advanced pipeline bispecific is a TA-MUC1-targeting T-cell engager (PankoMab-CD3-GEX)," Jäkel points out. "It was designed to combine the high tumor specificity of Gatipotuzumab with activation of polyclonal T cells independent of MHCI engagement upon simultaneous binding of TA-MUC1 and CD3 on T cells."

A second molecule in development at Glycotope is PankoMab-PDL-GEX, which combines binding to TA-MUC1 with immune checkpoint molecule PD-L1 attached to a glycol-optimized functional Fc domain. PankoMab-PDL-GEX is designed to direct checkpoint blockade to the tumor and thereby enhance tumor cell killing.

Glycotope's GlycoExpress (GEX®) technology platform is used for screening and production of biopharmaceuticals, such as those described above, and other glycoproteins for fully human optimized glycosylation. "It consists of a toolbox of proprietary human cell lines generated by glycoengineering," says Jäkel. "It is biotechnologically optimized for product improvement as well as fast, reproducible, and high-yield glycoprotein production."

"We do not use a standard platform approach for our bispecific programs," Jäkel continues, suggesting that by focusing on GlycoTargets, the company has positioned itself to screen several construct formats for each bispecific product idea. "We can produce classical IgGs but also bispecific formats in our GlycoExpress system," she asserts. "We can test different glycosylation variants for identification of a lead candidate with highest antitumor efficacy."

There are many possible advantages to targeting two epitopes, including greater specificity, increased inhibition of tumor growth, and enhanced local tumor cell killing.

Although Glycotope is not exclusively focusing on the bsAb market, Jäkel suggests that there are many possible advantages to targeting two epitopes over monospecific antibodies, including increased specificity and/or avidity, increased inhibition of tumor growth, enhanced local tumor cell killing, and blockade of immune checkpoint inhibitors.

Beyond bispecifics

In immuno-oncology, a well-trod path is the redirection of tumor T cells. A less-well-traveled path is being explored by **Invenra**, which seeks to activate functional processes that require a novel mechanism of action through bispecific and higher-order antibody binding.

"A good example is agonist antibodies for the tumor necrosis factor [TNF] receptor superfamily," says Bonnie Hammer, PhD, vice

president of biologic development at Invenra. "The ligands for that family are trimeric. To get good activity, you need at least three receptors coming together, but it is even better if you have even higher-order clustering."

Antibodies that drive this type of receptor clustering are the focus of Invenra's ARCHER (Agonistic Receptor Clustering by High-order Exogenous Rearrangement) technology. One of the receptors in the TNF superfamily, OX-40, is the target of an Invenra bsAb in lead selection.

To engage the higher-order clustering, Invenra used its B-Body multispecific antibody development platform to produce a bispecific with a two by one (2 × 1) format. "The bispecific has three Fab domains," Hammer notes. "But two Fab domains bind to one epitope, and the other Fab domain binds to a different epitope."

"Traditional monoclonal antibodies for OX-40 have suffered in the clinic," Hammer says, pointing out that they are dependent on having Fc engagement to provide the secondary crosslinking needed for activity. In contrast, she continues, Invenra's OX-40 agonist has allowed the company "to achieve activity in the absence of any additional crosslinking by targeting multiple epitopes." Although the OX-40 agonist has yet to see the clinic, Hammer suggests that the agonist "will provide higher activity than has been previously seen with monospecific antibodies."

A bacteriophage library that consists of wholly human Fab fragments and that matches the natural diversity found in the human repertoire can provide the starting point for selecting Fabs of interest used in Invenra's B-Body platform, Hammer says. A domain-substitution strategy with a few orthogonal chain mutations allows for highly specific light chain–heavy chain pairing and enables high-throughput production and purification of bispecific and multispecific antibodies.

"We found that you can predict some things [during antibody design]," she reports, "but a lot of it is through empirical testing. The affinities for the antibodies, the geometry, and the epitopes that you're hitting matter." One other group of multispecific antibodies in Invenra's pipeline consists of discovery candidates that create higher specificity through the targeting of more than one antigen. "These candidates are the bispecific antibodies we call the SNIPERs™," says Hammer. Currently a regulatory T cell–depleting SNIPER molecule is in lead selection. GEN

Optimizing Immunotherapy Lead Selection

By MaryAnn Labant

Chimeric antigen receptor (CAR) T-cell and bispecific antibody immunotherapies hold enormous potential for the treatment of cancer. But not all patients benefit from these therapies, especially those with solid tumor indications. An urgent need exists for novel detection strategies to better understand mechanistic function and efficacy at the single-cell level in a way that correlates to clinical outcomes. Population level studies do not provide the necessary insight into immune response heterogeneity at the single-cell level.

IsoPlexis' single-cell proteomics systems address this challenge by connecting each immune cell to the many cytokines they secrete, which orchestrate the immune system. This cellular response sensitivity is used to discover correlations to in vivo events in immunotherapy patients.

The Polyfunctional Strength Index

The IsoPlexis system identifies which cells are polyfunctional, i.e. those powerful cells that secrete multiple cytokines and quantitates the cytokine concentrations from each cell. The Polyfunctional Strength Index (PSI) multiplies the number of cytokines secreted per cell with the amount of each cytokine, to identify highly potent immunotherapies.

PSI helps researchers better understand how T cells functionally respond to immunotherapies. It has provided biomarkers and mechanistic insights over the past year to improve the decision-making process for choosing CAR T-cell and bispecific antibody lead candidates.

"The ability to easily perform single-cell proteomics provides a way to understand the complexity of the cellular response to immunotherapy and then to design and test new therapies to enhance efficacy," said Charles Sentman, PhD, Director, Center for Synthetic Immunity, Professor, Geisel School of Medicine, Dartmouth College.

Using the IsoPlexis single-cell proteomics system, researchers have identified cytokine-based biomarkers, which objectively evaluate the quality of antitumor activity of CAR T-cell response (pre-therapy) in a manner that correlates with outcome. Findings like this will enable more precise medicine and improve patient results.

As bispecific antibodies, which bind to both T cells and tumor cells to help selectively kill cancer cells, share many similarities with CAR T-cell therapies in terms of tumor targeting (such as polyclonal T-cell activation, killing mechanisms, and cytokine-based toxicities), similar insight from the use of the IsoLight platform is expected in bispecifics as well.

Using PSI to Evaluate the Quality of Bispecific Antibodies

More than 90% of human tumors express ligands called natural killer group 2, member D (NKG2D) ligands (MICA, MICB, ULBP1-6), whereas very little is found in normal tissues. To explore whether targeting these antigens can enhance antitumor T-cell responses, bispecific T-cell antibodies were engineered by the Sentman Laboratory for MICA and NKG2D: B2-OKT3 (MICA x CD3) and hNKG2D-OKT3 (NKG2D ligands x CD3).

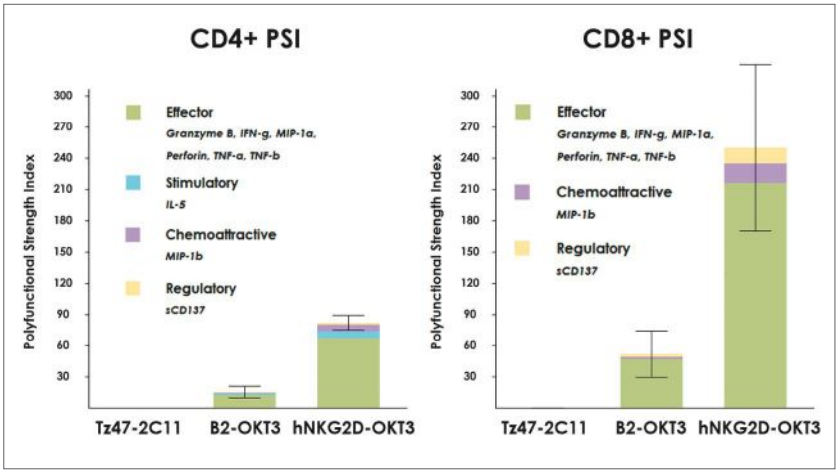
To test whether the engineered bispecific constructs could induce a potent antigen-specific T-cell response, single-cell PSI across five functional cytokine groups, effector, stimulatory, regulatory, inflammatory, and chemoattractive, was investigated.

The results showed that hNKG2D-OKT3 bispecifics induced a more potent polyfunctional T-cell response than B2-OKT3 bispecific proteins from both $CD4^+$ and $CD8^+$ human T cells (see *Figure*). Both profiles were dominated by effector cytokines. $CD4^+$ and $CD8^+$ cells show the same trend and almost identical protein secretions. NKG2D can recognize multiple ligands, including MICA, MICB, and ULBP1-6, whereas B2 binds only MICA. The results suggest that bispecifics capable of binding to multiple tumor antigens may yield better therapeutic effect. Tz47-2C11 was the negative control protein.

Visualizing Cellular Heterogeneity to Determine Optimal Bispecifics

The IsoLight's plug-and-play system includes a fully automated workflow and bioinformatics pipeline. Unique data visualizations, such as functional heat maps, polyfunctional activation topology principal component analysis (PAT PCA), and rank-ordered PSI bar graphs, are presented to explore and characterize single-cell polyfunctional profiles and reveal cellular insight across samples.

IsoPlexis' single-cell system improves insights into mechanism at the early stages of discovery by evaluating the quality of bispecific constructs and CAR T-cell products in a scientifically rational manner, offering a new approach for optimal lead selection. ■



Bispecific T-cell antibodies were engineered by the Sentman Laboratory for MICA and NKG2D: B2-OKT3 (MICA x CD3) and hNKG2D-OKT3 (NKG2D ligands x CD3). To test whether the engineered bispecific constructs could induce a potent antigen-specific T-cell response, single-cell PSI was computed for $CD4^+$ and $CD8^+$ T cells. The figure shows that hNKG2G-OKT3 induces more potent upregulation of polyfunctional strength index (PSI) than B2-OKT3 in both $CD4^+$ and $CD8^+$ T cells by the activation of K562 cells. Tz47-2C11 is the control. $CD4^+$ and $CD8^+$ cells show the same trend, with both profiles dominated by effector cytokines secretions.

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Compound Library Profiling of Immunomodulators

Evotec and Intellicyt describe multiplexed measurements of cell phenotype, viability, and cytokine secretion of human PBMCs

Serge P. Parel, PhD, Laurent Brault, PhD, Tom Duensing, PhD, Zhaoping Liu, PhD, and Daniela Brodbeck, PhD

Recent successes of immunomodulatory approaches for the treatment of serious diseases such as cancer have generated a significant growth in efforts aimed at the discovery of novel therapeutics in this area. Immunomodulatory agents are expected to affect interactions among cells and signaling molecules involved in regulating the immune system. Therefore, hit discovery campaigns need to deliver profiles of the impact of compounds on these complex interactions.

Human peripheral mononuclear cells (PBMCs) are a primary source of various immune cells, including but not limited to NK cells, B cells, and T cells of various subtypes and stages of differentiation. PBMCs are an excellent model system for studying effects of potential drugs on the immune system, as many of the modulatory effects of compound treatment can be recapitulated. In this complex cell mixture, activation or suppression of the immune response (immunomodulation) is often seen in concert with coordinated cytokine secretion patterns (*Figure 1*).

T cells can be activated by treatment with phytohemagglutinin (PHA), which will trigger proliferation of the T-cell population as well as modulate the cytokine secretion profile of a PBMC culture as a whole. T cells are identified by the surface marker CD3. A specific subtype, cytotoxic T cells (CTLs), will also express CD8. Compounds that alter the ability of PHA-stimulated T cells to proliferate or secrete certain cytokines might be candidate immunomodulatory compounds for further investigation.

Library biased toward immunomodulating compounds

Based on a literature search for substances having a differential effect on cytokine secretion, seven compounds were selected (imiquimod, mycophenolic acid, resveratrol, thalidomide, tomentosin, verapamil, and compound C3) and used as templates for running an extensive structure–activity relationship (SAR) expansion against the Exquiron Compound Collection of 260,000 compounds, based on a published protocol.[1] After a data fusion step, a total of 1438 compounds were identified. These compounds were cherry-picked for testing at two different concentrations (16.7 and 83 µM).

No-wash screening workflow

PBMCs were batch-labeled with the MultiCyt® FL4 Cell Proliferation Dye before plating into 384-well plates containing compounds from the SAR expansion selection. Each plate also included four reference substances as dilution series (resveratrol, verapamil, dexamethasone, and mitomycin C). Following plating of cells, PHA was added and cells were incubated for 3 days under appropriate tissue culture conditions (*Figure 2*).

After incubation was complete, 10 µL aliquots were stamped from each treatment plate into a multiplex of immunophenotyping antibodies (anti-CD3-FITC and anti-CD8PE) and the MultiCyt FL3 Membrane Integrity Dye. A second stamp of 3 µL from the same motherplate was used for QBeads detection of interleukin-17f (IL-17f), IL-6, and tumor necrosis factor (TNF). Each plate was read on

Figure 1.

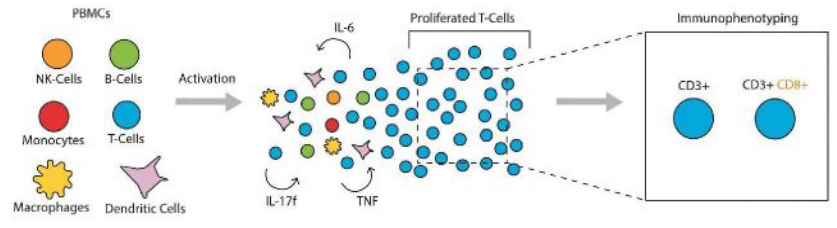
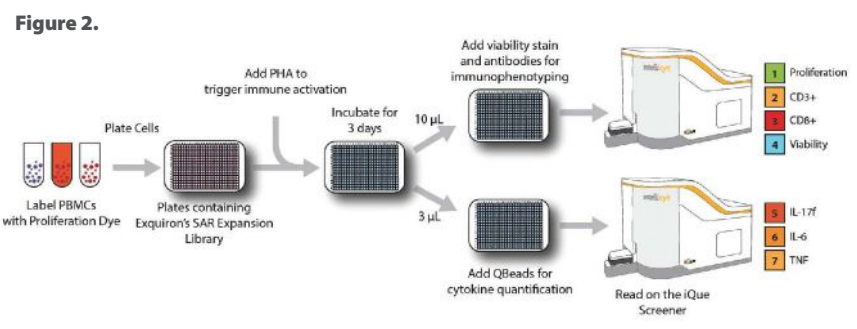


Figure 2.



the iQue Screener immediately after staining, without wash steps. Each 384-well plate took about 25 minutes to read.

Data analysis and activity profile generation

Among all the data generated by the iQue Screener, 11 parameters were extracted based on their biological significance (*Table*).

Data were normalized plate-wise to the PHA-activated control cell population, using a modified *z* score transformation, and activity profiles were generated.[2] Profiles for resveratrol at various concentrations are shown below (*Figure 3*).

Identification of compounds inducing specific phenotypes

Calculation of the Euclidian distance between profiles and subsequent similarity search against the profiles of the reference substances allowed the identification of compounds displaying specific phenotypes (Cpds1–4 and Cpds5–9 induce dexamethasone-like and verapamil-like phenotypes, respectively) (*Figure 4*).

Identification of compounds displaying new phenotypes

Through clustering and subsequent visual inspection of the activity profiles, compounds eliciting new phenotypes (that is, phenotypes not covered by the controls or reference substances) were identified as well. Examples for compounds inducing TNF s ecretion is shown in *Figure 5*.

Summary

High-throughput, multiplex screening of compounds on primary cells generates information-rich multivariate compound activity profiles that can be used for identifying or prioritizing potential therapeutic candidates.

Application of advanced data mining techniques to these profiles allows for the rapid identification of compounds with activities similar to those of reference substances (potentially bridging the gap between phenotype and mechanism of action), but also identifies compounds eliciting new, potentially interesting phenotypes. GEN

Index	Population	Parameter
1	Total cells	% proliferated cells
2		% viable cells
3	Cytotoxic T-cells ($CD3^+/CD8^+$)	% of cytotoxic T-cells (CTL)
4		% viable CTL
5		% proliferated CTL
6	Other T-cells ($CD3^+/CD8^-$)	% of non-cytotoxic T-cells (nCTL)
7		% viable nCTL
8		% proliferated nCTL
9	Secreted cytokines	IL-6 (median FL2-H)
10		TNF (median FL2-H)
11		IL-17f (median FL2-H)

Table.

Serge P. Parel, PhD, is director, chemistry and research informatics, Laurent Brault, PhD, serves as project leader assay development and hit discovery, and Daniela Brodbeck, PhD, works as director, biological sciences at Evotec. Website: www.evotec.com.

Tom Duensing, PhD, is chief technology officer and Zhaoping Liu, PhD, is senior scientist at IntelliCyt. Website: www.intellicyt.com.

References available online.

Figure 3.

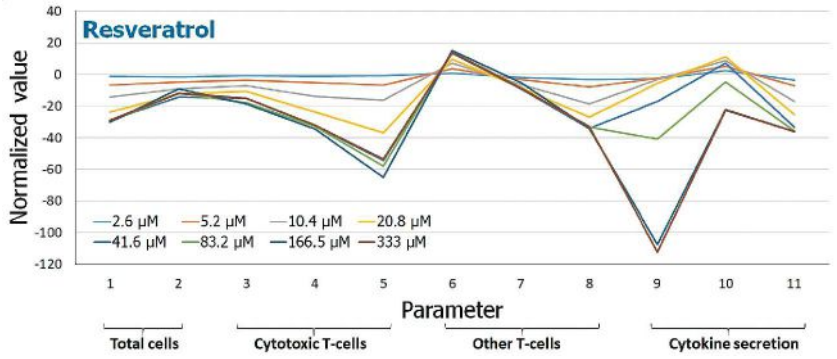


Figure 4.

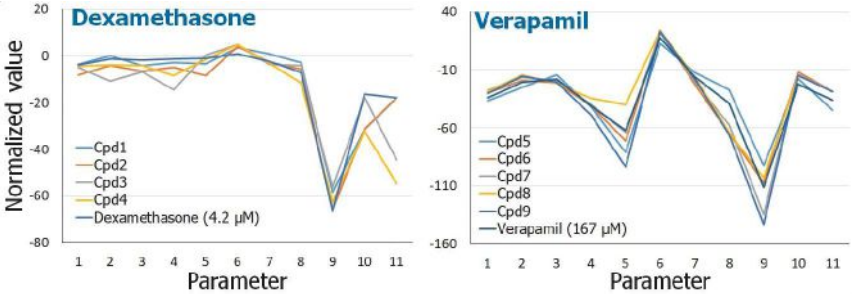
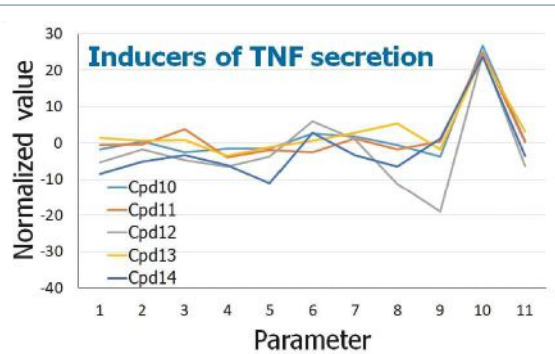


Figure 5.



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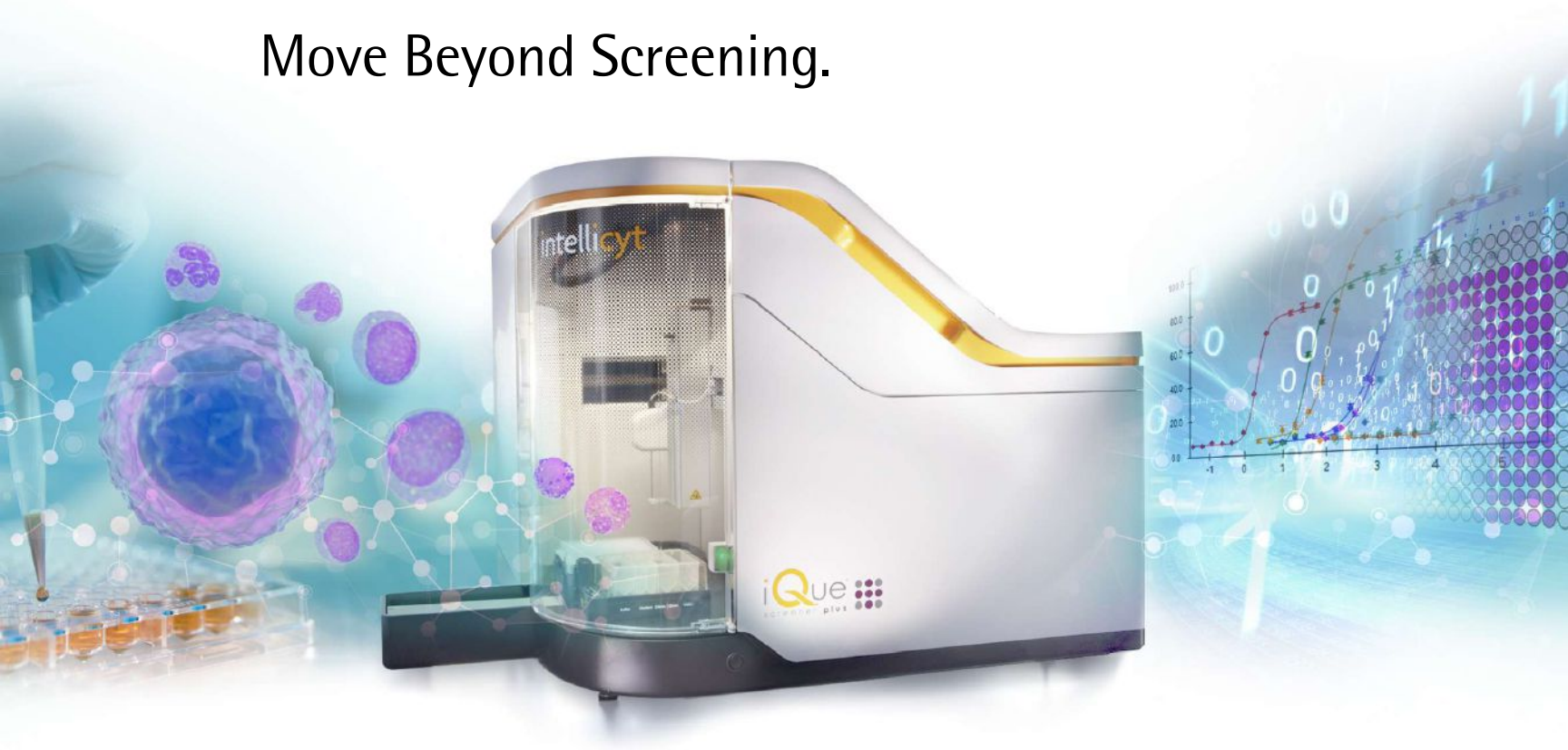

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Considerations in Drug Screening Assay Development

PerkinElmer takes a comprehensive view of the experimental factors that must be optimized to ensure developmental success

Catherine Lautenschlager, PhD, Roger Bosse, PhD, Jen Carlstrom, PhD, and Anis H. Khimani, PhD

Optimization of experimental assay design is crucial for the overall success of any drug discovery initiative. The factors that need to be considered include: selection of the most appropriate assay technologies, reading modalities, and experimental models (biochemical, cellular, or animal). Collectively, these factors are known to influence data quality, biological relevance, and therapeutic predictability, which will ultimately impact the success of entire preclinical drug development efforts.

Assay technology selection

Biological relevance and therapeutic predictability

The first step in selecting an assay technology is to question the paradigms to be addressed. Some examples are:

- Activation, inhibition, or modulation of a target
- Elucidation of MOA (mechanism of action)
- Determination of binding affinity of receptor–ligand or protein–protein interactions
- Identification and quantitation of disease-specific biomarker or biomarker panel in plasma

Defining the paradigms and predictable end point(s) enables selection of the assay formats and technologies to be used for the study.

The assay technology selected needs to reproduce physiologically relevant conditions; that is, it needs to be performed in relevant in vitro systems with pharmacologically relevant concentrations of components to yield biologically relevant data. Phenotypic cellular assay models more accurately reflect the complex biology of disease, allows for multiparametric measurement of ex vivo or in vivo events, and enables the characterization of intractable target(s).

However, such assays sometimes require complex execution, which can impede the screening process. Biochemical assays are simpler to execute than their cellular counterparts and offer better consistency. Unfortunately, the latter don't guarantee high physiological relevance. Hence, it is desirable to start with a biochemical assay followed by a cell-based assay to corroborate results in a physiological context (that is, to provide orthogonal validation).

Sample matrix compatibility

Biomarker assays aim to detect or quantitate targets in biological samples (for example, complex matrices) ranging from secreted proteins in cell culture supernatants to cell lysates, tissue extracts, saliva, urine, serum, plasma, blood, or other fluids (for example, CSF, BALF). Assay technologies may not always be compatible with particular matrices, which can cause interference. For example, hemoglobin found in serum, plasma, or blood exhibits wide absorbance across the visible light spectrum, namely between 350 and 600 nm. Assay technologies relying on excitation or emission at these wavelengths will be prone to interference. Wash-based assays can be used to circumvent interference caused by highly complex sample types, as molecules within the matrix that contribute to interference are washed away. To determine sample matrix compatibility, spike-and-recovery and linearity experiments are traditionally performed in the appropriate diluent for standard curves.

Additionally, for complex biological matrices, antibody selection can be challenging since protein targets secreted from cells into cell culture media or serum may be cleaved or modified, altering epitope recognition—hence the benefit of using protease cocktail inhibitors.

Assay performance

Sensitivity

Sensitivity of an assay dictates the detection of the target within the dynamic range of the assay with the caveat that the lower limit of detection could vary marginally from batch to batch.

Sensitivity is also dependent on sample type. Physiological levels of an analyte in serum differ from the levels secreted by a given cell line. "Precious" samples, such as limited cells or tissue, impact the permissible volume for testing. Technologically, luminescent and fluorescent modes offer greater sensitivity compared to absorbance assays. Both assay format and detection technology play major roles in sensitivity.

Dynamic range

The dynamic range of an assay defines both the lower and upper limits of detection. For example, as shown in *Figure 1A*, the dynamic range for quantitating tumor necrosis factor-α (TNFα) varies by at least a log across different technologies. Furthermore, measurable binding affinities for various analyte interactions can vary based on the chemistry and detection technology (*Figure 1B*). Therefore, when samples fall outside the limits of detection of an assay, concentrations of analyte, kinetic parameters, potencies, and binding affinities could result in variations of measurement. Hence, cell or analyte titrations and calibration curves are implemented to ensure detection windows and avoid saturation.

Specificity

Specificity is important to ensure that the assay will measure the desired target or phenotype. For immunoassays, the appropriate antibody-to-target-analyte specificity needs to be considered especially since targets may vary from modified moiety or cleaved form (neoepitope) of a protein to bound/unbound or active/inactive forms. In addition, species and target cross-reactivity need to be considered.

For assays involving conjugation or labeling of antibodies, specificity should be confirmed. For biochemical enzymatic assays, substrate selection is important to achieve specificity.

For example, many recombinant, purified protein kinases can be used with generic or specific peptides, or whole protein substrates. In addition, to prove specificity, reference inhibitors should be validated for the developed assay.

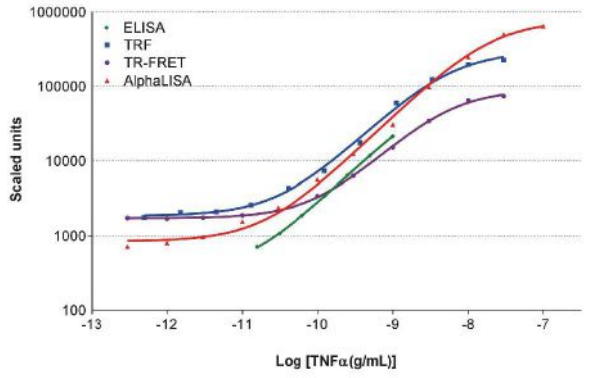

Figure 1A. Dynamic range and sensitivity of TNF-α quantitative immunoassay using four different assay technologies.

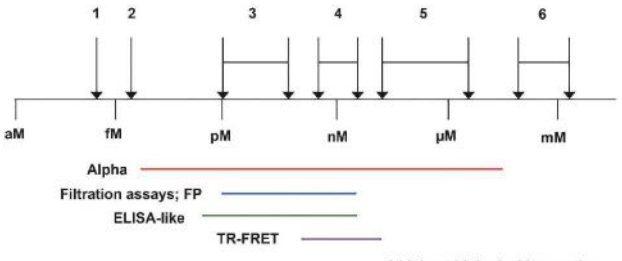

Figure 1B. Detectable/measurable affinity ranges for binding assays for four different assay technologies. Affinity defined by K_d of binding interaction.

1 highest biological interaction
2 biotin-streptavidin
3 very high affinity ab, receptor ligands
4 most antibodies of good quality
5 most protein-protein interactions
6 lectins

Robustness and accuracy

Although signal intensity or signal-to-background ratio (S/B) of an assay is typically evaluated, it is vital that the assay is robust enough to yield highly reproducible and accurate data. For instance, the *Z*-prime value (*Z*') is usually a better indicator of robustness compared to S/B. For example, a low signal intensity (low S/B) but a high *Z*' assay is better than a high signal (high S/B) but a low *Z*'. Also, the assay should be highly reproducible, delivering the same quality data irrespective of the day or the individual running the assay. Quantitative assays should be calibrated against an appropriate standard. Reliability of an assay can be achieved using internal and external controls and an array of reference calibrators, antibodies, and compounds as appropriate.

Semiquantitative or qualitative assays should be run using reference compounds or binding molecules as appropriate. Acceptable levels of robustness, reproducibility, and accuracy are dictated by the goals of the assay and must be determined by the researcher.

Reagents and consumables

Reagent quality

For immunoassays, one must consider the selection of quality antibodies based on their sensitivity, specificity, and continuous availability (that is, reproducible production). Relying on monoclonal antibodies (mAbs) guarantees the latter aspect. Similarly, validated high-quality enzymes and recombinant proteins should be used for developing biochemical enzymatic and binding assays.

Reagent titration and optimization

In general, all reagents (such as cells, proteins, enzymes, cofactors, substrates, stimulators, inhibitors, agonists, and antagonists) utilized in an assay need to be titrated in cross-titrations or stepwise via optimal concentrations. The process ensures steady-state kinetics

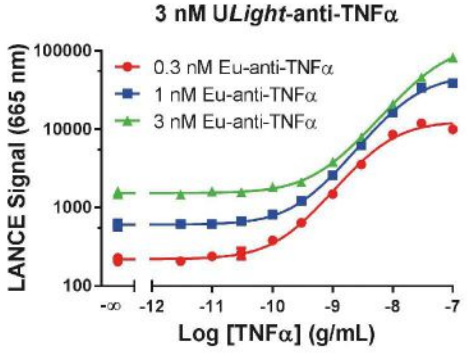

Figure 2. Titration of europium-labeled anti-TNF-α detection antibody in a LANCE TR-FRET assay for TNF-α detection.

and prevents saturating concentrations to maximize S/B while reducing reagent consumption (*Figure 2*).

Cell models

When choosing cellular models, one should consider the advantages and disadvantages of using primary cells vs. recombinant/immortalized cell lines, and the study of endogenous vs. recombinantly expressed target proteins. The target expression level needs to be assessed, as too much or too little expression can affect sensitivity and assay quality. Cell passaging and culture conditions can also affect functional response.

Microplate selection

Correct selection of a microplate can improve assay performance by reducing crosstalk, decreasing background, minimizing signal absorbance, and/or allowing for signal amplification. The *Table* shows a matrix for microplate selection based on the method of detection.

Summary

Drug discovery initiatives are known to be costly and time consuming. Optimizing experimental factors known to influence data quality, biological relevance, and therapeutic predictability ultimately drives the success of entire preclinical drug development efforts. Selecting the most appropriate assay technologies, reading modalities, and experimental models are examples of experimental factors to optimize for laying the groundwork of successful and cost-effective drug development. GEN

Catherine Lautenschlager, PhD, is e-marketing manager, Roger Bosse, PhD, is sales development leader, Jen Carlstrom, PhD, is senior applications scientist, and Anis H. Khimani, PhD, is director, strategy leader and applications, for PerkinElmer's Discovery & Analytical Solutions business. Website: www.perkinelmer.com.

Table. Microplate selection* based on detection method

Detection Method	Recommended plate material	Recommended plate color
Absorbance/Colorimetric	**Wavelength-dependent material** **Visible light range (400–900 nm wavelengths):** Polystyrene plates **Ultraviolet range (200–400 nm wavelengths):** UV-transparent material (e.g., glass or COC) to avoid autoabsorbance of light by plastic	Clear or clear-bottomed with opaque walls to reduce crosstalk at 384-well and 1536-well densities
Fluorescence		
FP, FRET	Polystyrene	Black to reduce background autofluorescence
TR-FRET and TRF	Polystyrene	White (background autofluorescence already reduced by time resolution)
Luminescence	Polystyrene	**Black:** High-signal assays **White:** Low-signal assays **Gray:** Optimal performance
Alpha	Polystyrene	**White:** Maximal signal **Gray:** Reduce crosstalk
Radiometric	**GF/B, GF/C, or Nylon filter mats:** Cellular, cell membrane, nucleic acid filtration assays **Polyethylene plates:** Scintillation cocktails **Polystyrene plates:** General radiometric **Scintillant-coated polystyrene plates:** Proximity assays	White plates or plates with white well walls/clear bottoms for bottom reading
High-Content Screening	**Glass bottom:** Best optical properties, but not well suited for cell culture. **Tissue culture (TC)-treated polystyrene:** Supports cell attachment. (**Polystyrene plates are also offered with coatings to facilitate cell attachment.) **Cyclic olefin:** Supports cell attachment and growth while offering better optical properties compared to polystyrene. (**Cyclic olefin plates are also offered with coatings to facilitate cell attachment.)	Clear bottoms with black well walls to reduce autofluorescence

***For a list of microplates available from PerkinElmer, refer to:** https://www.perkinelmer.com/lab-products-and-services/application-support-knowledgebase/microplates/microplate-table.html

****Plate coatings for cell attachment:** Tissue culture-treated-plates or polylysine- or collagen-coated plates

Transferring Viral Vector Production from Plasticware to a Fixed-Bed Bioreactor

Proof-of-concept from Univercells for scalable HEK293 cell growth and adenovirus production

Yael Dohogne, Florence Collignon, Jean-Christophe Drugmand, Alex Chatel, José Castillo, Satoshi Kumano, and Hitoshi Shiomi

Gene and modified cell therapies (for example, chimeric antigen receptor [CAR] T-cell therapies) in early to late clinical development are showing significant promise to treat and potentially cure many diseases. A few of these next-generation drugs have already been approved and have reached the market. Despite the robust pipeline of candidates advancing through the clinic, however, there is potential for a manufacturing bottleneck—viral vector production—to delay commercialization.

Unlike most engineered protein-based biologics, which are manufactured using suspension cell culture, viral vectors require the use of adherent cells that are supported on a matrix. Two options are currently available for adherent cell growth: the use of microcarriers or multitray static plasticware.

Microcarriers are suspended in a tank and agitated, providing a homogeneous environment. The agitation can be too rough for the shear-sensitive cells required for viral vector production, however. Process scale-up requires significant effort because the hydrodynamic conditions within the reactor change. In addition, it is not possible to achieve high cell densities, and scale-up entails the use of large quantities of expensive transfection agent.

For these reasons, static plasticware has been traditionally used for adherent cell growth, transfection, and adenoviral vector production. However, this method also suffers from limitations. Precise environmental control (pH, dissolved oxygen [DO], media composition) is not possible, and extensive manual intervention is required. Furthermore, scale-up is not possible—only scale-out.

A scalable solution

Scalable and automated single-use fixed-bed bioreactor technology from Univercells addresses these shortcomings and offers drug manufacturers the opportunity to supply the market with the required quantities in an affordable manner. A schematic of the bioreactor is shown in *Figure 1*.

A tightly packed support matrix comprising spiral-wound, nonwoven polyethylene terephthalate (PET) fabric layers allows for high cell densities in a small-footprint bioreactor. A magnetic centrifugal impeller located inside the bioreactor provides two functions: good mixing to ensure even availability of nutrients throughout the fixed bed, and aeration through the creation of a "falling film" via the vessel headspace to increase the surface area available for gas exchange. The media is gently circulated across and through the cells, providing conditions similar to those present in static plasticware, but with a homogeneous environment both vertically and horizontally.

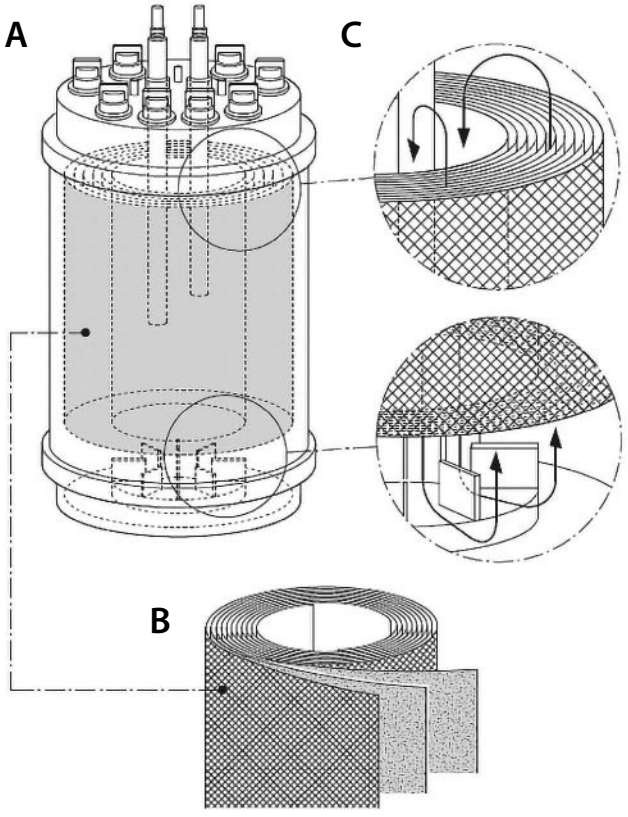

Figure 1. Schematic details of the scale-X carbo bioreactor. (A) Bioreactor vessel. (B) Structure of the fixed bed. (C) Fluid flow patterns within fixed bed.

The system includes automated pH, DO, and temperature control; automated inoculum addition and manual media and cell sampling; and pumps that allow further media addition through a recirculation loop.

Scaling is achieved by increasing the height with a fixed diameter (~10-, 20-, and 30-cm height of fixed bed), then increasing the diameter in parallel (10, 20, and 30 cm). Because the linear velocity of liquid media travelling through the fixed bed remains constant across scales, the cells experience similar low-shear conditions. The automation features and ease of scale-up facilitate more rapid development and commercialization of viral vectors.

Investigation of process transfer

The ease of transferring viral vector production processes from static plasticware to the fixed-bed bioreactor was investigated by comparing the performance of processes conducted in the scale-X™ hydro bioreactor system and a standard plastic flatware cell culture flask using the same inoculation density and proportional volume of culture medium for each.

The cell-culture process in the scale-X hydro system takes place in five steps: (1) bioreactor preparation, (2) inoculation and cell attachment, (3) cell expansion, (4) viral infection, and (5) harvest (*Figure 2*).

HEK293 cells from a cryopreserved cell bank (**Momotaro-Gene**) were used. The inoculum was generated in a cell culture flask under standard conditions. The processes were monitored for cell density and virus titer (visual counts using an optical microscope) and metabolite (glucose, lactose) concentrations (off-line metabolite analyser).

In all of the processes, an external media source was connected during cell expansion and recirculated through the bioreactor shortly after inoculation (*Figure 2*). In the first bioreactor (#1), the media was replaced at day 3 to allow further cell growth, but the cells were not infected. For bioreactors #2 and #3, cell expansion was performed in batch mode for 2 and 3 days, respectively, after which time the cells were infected. Although the cells naturally lyzed upon infection, product recovery was enhanced using detergent.

Successful cell growth and infection

Cell growth in the fixed-bed bioreactors (#2 and #3) was higher under the same conditions than that in the standard plasticware. (The results are presented in *Figure 3*.) It can be seen in the figure that the fixed-bed bioreactor outperformed the standard plasticware both at day 3 (2.1×10^5 vs. 1.3×10^5 cells cm^{-2}) and at day 6 (6.5×10^5 vs. 3.2×10^5 cells cm^{-2}). Note that the same ratio of medium exchange was used in the plasticware and bioreactor to achieve the high cell densities observed at day 6.

All bioreactors were operated with an external medium circulation loop of 4.2 L from inoculation. At day 3, the external media circulation loop of bioreactor #1 was replaced with fresh medium, thus allowing further cell growth until day 6. Bioreactors #2 and #3 were operated in batch mode and then infected (respectively at day 2 and 3). Cell density post-infection is not shown. Control cell density for bioreactor #2 was not available.

Infection was performed at a target cell density (days 2 and 3 in bioreactors #2 and #3, respectively), and all cells were observed to be lysed 3 days post-infection. The product from each process was then recovered using the same detergent treatment. The viral titer was approximately 1 log IRU less in the bioreactor than in the control plastic flatware without performing any process development work.

Potential for further improvement with process optimization

This study demonstrated that an HEK293 process producing an adenovirus for gene therapy based on the use of static plasticware

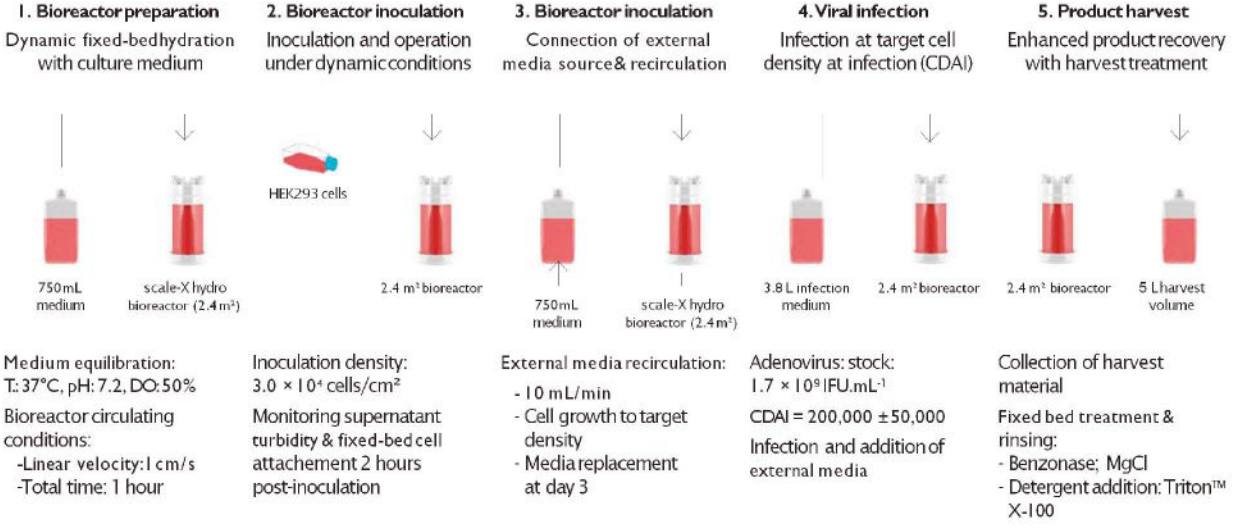

Figure 2. Process flow diagram of the experimental setup.

can be successfully transferred to the Univercells scale-X bioreactor system. The potential to reach very high cell densities and good viral yields within a small bioreactor volume highlights the system's capability for high levels of production in a much-reduced footprint, and with an automated process step.

Notably, the processes in the static plasticware and the fixed-bed bioreactor gave similar results without any effort to optimize the process performed in the bioreactor. The higher cell densities obtained in the scale-X hydro bioreactor are most likely a reflection of the better environmental control (pH and DO) in the system, which promotes a stable growth environment. Combined with the promising viral yields, the higher cell densities open the door for further process development.

Future work will focus on determining the critical process parameters (CPCs) for optimization of the culture conditions in the bioreactor, a feat that is not possible with traditional static processes due to the lack of control of process parameters such as pH and DO.

The scale-X hydro system is part of a portfolio of bioreactors allowing process development and pilot scale cultures (scale-X hydro at 2.4 m^2 and carbo 10–30 m^2), medium-to-large-scale industrial production (scale-X nitro, 200–600 m^2, typically suitable for vaccine production), and larger scale industrial production (scale-X oxo, >2000 m^2, to meet the needs of gene therapy). Further experiments are also planned to demonstrate the scalability of the process to these larger scale bioreactors. GEN

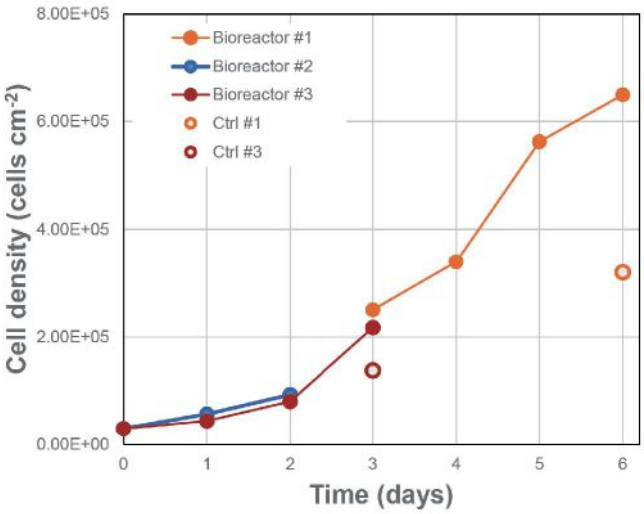

Figure 3. Comparative growth curves of HEK293 cells grown in scale-X hydro bioreactor and plastic flatware controls.

Yael Dohogne, Florence Collignon, Jean-Christophe Drugmand, Alex Chatel, and José Castillo are at Univercells (www.univercells.com). Satoshi Kumano and Hitoshi Shiomi are at Momotaro-Gene (www.mt-gene.com).

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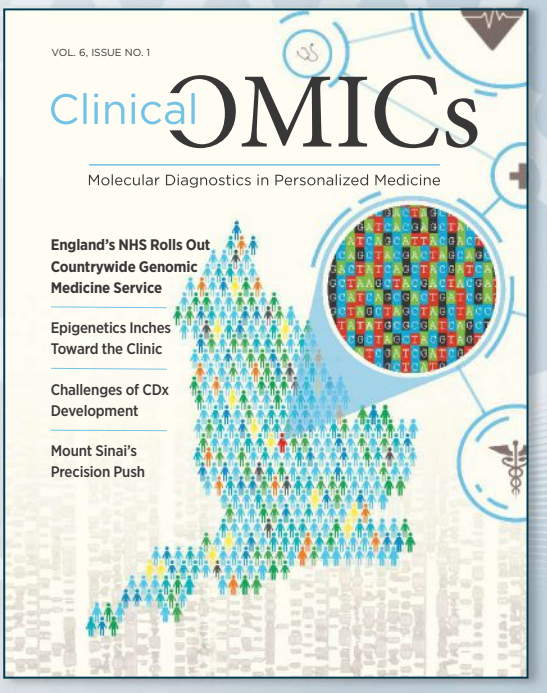

Microplate

The P3 microplate can be used to accelerate the removal of interfering proteins from serum, plasma, or even whole blood samples prior to analysis by LC–MS. Biological samples commonly contain proteins that interfere with downstream applications. Traditionally, scientists have used the CRASH method, in which the protein is denatured with acetonitrile and the flocculant filtered out to clean their samples prior to LC–MS analysis. Using a P3 protein "crashes" out of solution and precipitates directly in each well of the microplate when acetonitrile is added, thus solving all common problems traditionally associated with the CRASH technique of protein clean-up. The novel dual frit, hydrophobically treated matrix used by the P3 microplate means that there is no "wetting out" and leakage of the sample before the application of vacuum. The P3 microplate enables 96 samples to be filtered simultaneously—accelerating LC–MS protocols.

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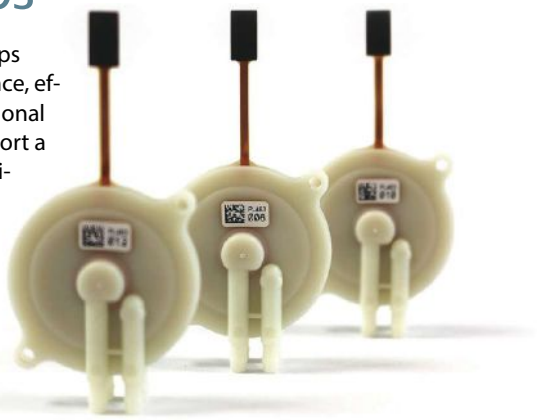
Micropumps



The XP Series of micropumps offers enhanced performance, efficiency, and a wide operational temperature range to support a more diverse range of applications across the medical, life sciences, environmental, and defense sectors. The Disc Pump platform delivers high pressure and flow, silent operation, rapid response time, accuracy, infinite turn-down ratio, and ultra-smooth flow in a compact package, to support product innovations through wearability, portability, and simplicity. The XP Series is a new addition to this range, providing up to 50% increase in peak efficiency, up to 30% increase in pressure and flow, and enhanced stability across a wider operating temperature range of –25 to 55°C. This was achieved through a combination of design alterations, new materials, and manufacturing process development, meaning the XP Series enables longer battery life, operation in harsh environments, and longer operational life.

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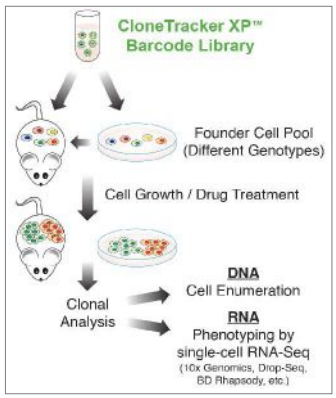
Cell-Labeling Libraries



The CloneTracker XP™ Expressed Lentiviral Barcode Library, and Barcoded CRISPR Library product lines feature the ability to label and trace individual cells. Together with sequencing technology, they enable a new approach for tracking clonal variations in large cell populations and can be powerful experimental tools in many research areas, including cell development and evolution, stem cell biology, or carcinogenesis. The new Barcode Libraries' unique DNA sequence (i.e., the barcode) is designed to express on an RNA transcript in the cells. As a result, it can be detected by either DNA or RNA sequencing. A variation of the new CloneTracker XP barcode labeling product is also available that offers a gene effector, in this case, CRISPR sgRNA, into the barcode library. Each effector targets and disrupts a specific gene in each of the cells that pick up a barcode. In combination with cell-specific barcode tracking, this knockout enables researchers to see how particular genetic disruptions change the characteristics of the cells, while simultaneously also identifying the genetic pathways that are activated to produce them.

Cellecta
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Zika ELISA Assays

This new series of Zika virus assays have little or no cross-reactivity with other flaviviruses. The Zika Virus NS1 ELISA assay is a highly sensitive and specific assay suitable for the quantitative detection of Zika Virus NS1 in a range of biological samples. This ELISA assay can detect NS1 antigen at levels as low as 5 pg/mL and enables researchers to measure the level of Zika NS1 protein in infected patients, offering value in epidemiology studies. The Zika Virus IgG/IgM/IgA ELISA assay is designed for the detection of Zika-specific antibodies in human serum. It is minimally cross-reactive with antibodies to Dengue virus and so can be used to distinguish human anti-Zika antibodies from other flavivirus and infectious disease antibodies. This assay has a short assay time of only two hours, with specificity and sensitivity both in excess of 90%, even in regions endemic with Dengue. Due to the novel assay format, the test may also be used in any species, making it of value for studying animal models of Zika infection.

The Native Antigen Company
www.thenativeantigencompany.com

FEBRUARY

February 19–21
Disposable Solutions for Biomanufacturing Global, Amsterdam, The Netherlands. www.disposablebiomanufacturing.com.

February 20–21
3D Cell Culture, in London, U.K. www.3d-cellculture.com/GEN.

February 21–22
Biomarkers Congress, Manchester, U.K. www.biomarkers-congress.com.

February 26–28
AsiaTIDES, Tokyo, Japan. lifesciences.knect365.com/asia-tides.

February 27–March 2
AGBT, Marco Island, FL. www.agbt.org/the-general-meeting.

MARCH

March 5–6
BIO Asia International Conference, Norfolk, VA. www.bio.org/events/bio-asia-international-conference.

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March 11–14
BioProcess International West, Santa Clara, CA. lifesciences.knect365.com/bpi-west.

March 14–15
Future of Individualized Medicine, La Jolla, CA. www.scripps.edu/science-and-medicine/translational-institute/about/events/foim.

March 14–15
Next Gen Immuno-Oncology Congress, London, U.K. mnmconferences.com/3rd-Annual-Marketsand Markets-Next-Gen%20 Immuno-Oncology-Congress.

March 12–19
AT Europe, Dublin, Ireland. www.casss.org/page/ATE1900.

March 17–21
Pittcon, Philadelphia, PA. www.pittcon.org.

March 18–19
Drug Discovery Chemistry, London, U.K. www.drug-discovery.co.uk/GEN.

March 18–20
Phar-East, Singapore. www.terrapinn.com/exhibition/phar-east.

March 25–27
Bio Europe Spring, Vienna, Austria. ebdgroup.knect365.com/bioeurope-spring.

March 27–29
Circulating Biomarkers World Congress, Boston, MA. selectbiosciences.com/conferences/index.aspx?conf=CBWC2019.

March 29–April 3
AACR Annual Meeting, Atlanta, GA. www.aacr.org.

COMPANY INDEX

Adel Nada, MD
CASEBIA THERAPEUTICS

Nada was hired as the company's first CMO. He is the former vice president, immunotherapy, at Caladrius Biosciences of New York (previously known as NeoStem). Before that, he was senior medical director of cardiovascular cellular therapies at Baxter Healthcare. Nada also held a variety of positions at Abbott Laboratories, including several as medical director.

Holger Wesche, PhD
HARPOON THERAPEUTICS

Wesche was promoted to CSO. He was svp, research; prior to that, he was a scientific director at Amgen. Before that, he held a leadership role, in drug discovery and cell-signaling research, at Tularik.

Ori Ben-Yehuda, MD, joined **CARDIOVASCULAR BIOTHERAPEUTICS** as CMO.

Ian J. Reynolds is the new CEO of **REWIND THERAPEUTICS**.

Natalie R. Sacks, MD, joined **HARPOON THERAPEUTICS** as CMO.

TAROS CHEMICAL added **Torsten Hoffman, PhD**, as svp, drug discovery.

VASOMUNE THERAPEUTICS welcomed **Douglas A. Hamilton** as president and CEO.

SPECTRUM PHARMACEUTICALS hired **Francois Lebel, MD**, as CMO.

KALA PHARMACEUTICALS welcomed **Steven Zhang, M.D., PhD**, as vp, medical affairs.

PHAGENOVA BIO hired **Renata Pasqualini, PhD**, as CSO.

Phil Jeffrey, PhD, joined **BICYCLE THERAPEUTICS** as svp, preclinical development.

SEMATHERA welcomed **Garth Cumberlidge, PhD**, as president, CEO, and member of the board of directors.

CHROMADEX hired **Matthew Roberts** as CSO and svp, innovation.

Joanne Kotz, PhD, joined **JNANA THERAPEUTICS** as CEO.

STRATA ONCOLOGY welcomed **Scott A. Tomlins, MD, PhD**, as CMO.

MPERIA THERAPEUTICS hired **Paul Chipperton** as president and CEO.

Julian Blagg, DPhil, joined **AZERIA THERAPEUTICS** as vp, drug discovery.

Karl Keegan, PhD, is the new CEO of **HOX THERAPEUTICS**.

AGILVAX appointed **Joseph Patti, PhD**, as executive chairman of its board of directors.

Niraj Vasisht, PhD, is the new president and CEO of **AVIOR BIO**.

REXAHN PHARMACEUTICALS promoted **Douglas J. Swirsky** to president, CEO, and member of the board of directors.

RAKUTEN ASPYRIAN hired **Mickey Mikitani** as CEO.

DERMAVANT SCIENCES welcomed **Todd Zavodnick** as CEO.

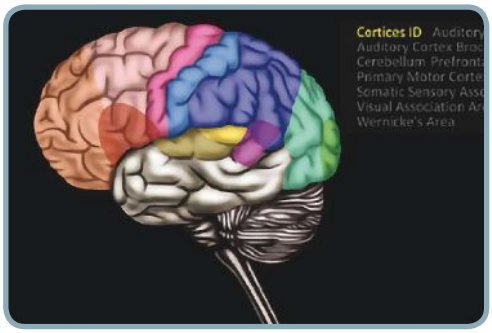
ADVERTISER INDEX

Classified

Pathway Tract ID ☆☆☆☆

Platform: iPhone/iPad **Android:** Yes **Cost:** $0.99

Easy to use, information included about many brain areas

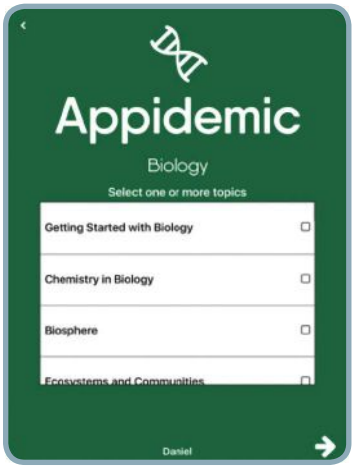


Pathway Tract ID is a fun and interactive tool for examining the fiber tracts that run through the central nervous system. Users can peruse through brain areas known to be involved in different behaviors, such as audition or movement. Clicking on a brain area brings up text describing its location, connections, and known functions. Users can also choose to watch short animations that show how information descends through the selected pathway. Additionally, the app allows users to take screenshots of their pathway of interest and even has a drawing feature that lets users annotate the images. Pathway Tract ID provides a fun and intuitive way for users to examine the brain more closely and discover how different parts of the brain interact with each other.

Appidemic Biology ☆☆☆☆

Platform: iPhone/iPad **Android:** No **Cost:** $1.99

Challenging and interesting questions and a range of topics



Appidemic Biology is a great app for students or anyone looking to refresh some of their general biology knowledge. Users can choose to challenge themselves in single-player mode or challenge other players in challenge mode. The app includes a wide range of biology topics that users can get quizzed on, ranging from the history of life to cellular respiration to the biosphere. Users can choose as many topics as they want to get quizzed on and can choose the number of questions that they are asked. The multiple-choice questions are timed and provide a fun challenge. The app also allows users to keep track of their high scores and compare with friends. Appidemic Biology is a fun app that doubles as a helpful study tool for students.

Virus Encyclopedia ☆☆☆

Platform: iPhone/iPad **Android:** No **Cost:** $1.99

Easy to browse through viruses

No external references provided

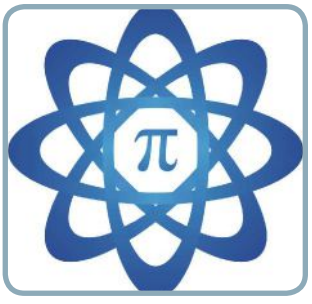
Virus Encyclopedia allows users to browse through an extensive list of known viruses. The app contains information about over 300 different viruses and organizes them by group for easy browsing. Each virus page contains information about the virus' family and genus, and a detailed description about the virus, including its incubation period, transmission mechanism, and symptoms that it causes. Some of the pages also include images of the viral particles. The app allows users to bookmark and take notes on particular viruses of interest right from the app. Additionally, a virus search feature makes finding known viruses easy. The Virus Encyclopedia is a great catalog of viruses for easy perusal right from your phone.

MathKit ☆☆

Platform: iPhone/iPad **Android:** No **Cost:** Free ($2.99 to unlock full functionality)

Easy to input values for variables, well organized by topic

Limited formulas included in the free app



MathKit is a handy app for doing all of your mathematical calculations on the fly. The app includes lists of common equations divided into two categories, math and physics. Each category lists out more specific available topics. For example, users perusing the math category can find equations for algebra, calculus, or coordinate geometry, while the physics category includes equations for force, light, motion, and thermodynamics. The app provides the full written equation and also allows users to input known values so that the app can solve the rest of the equation for you. Unfortunately, although the app itself is free, users have to pay to gain access to all of the equations available on the app. However, MathKit is still a convenient tool that provides a refresher on key formulas in math and physics and allows users to solve the equations right within the app.

Links to the apps described above are posted on GEN's website, **www.GENengnews.com**.
To suggest an app for Best Science Apps, please send a link to Kristen Drummey (**kristendrummey@gmail.com**).

RATINGS Excellent ☆☆☆☆ Very Good ☆☆☆ Good ☆☆

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