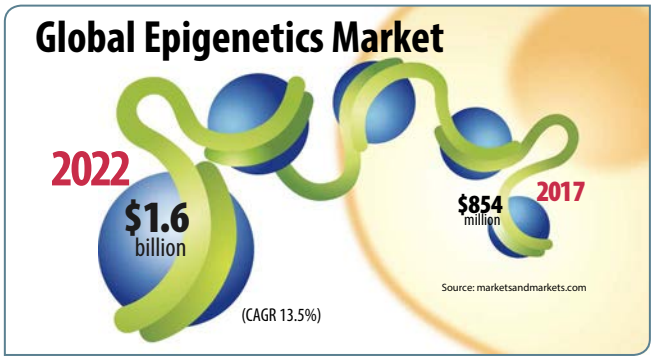
GEN Genetic Engineering & Biotechnology News



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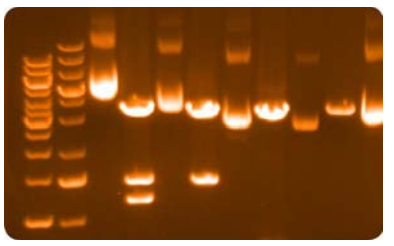
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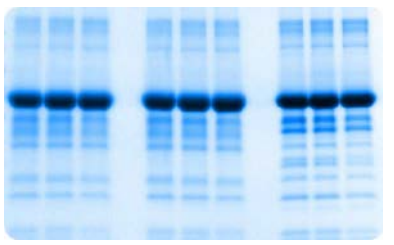
Protein gels


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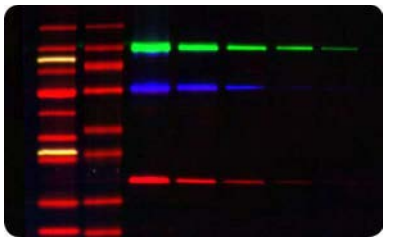
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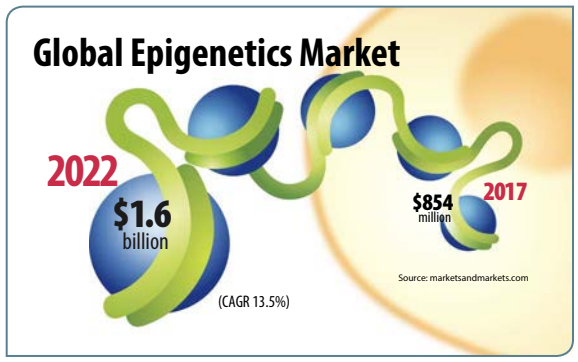


Epigenetics Revitalizes Drug Discovery

Julianna LeMieux, Ph.D.

In a relatively short time, epigenetics has transformed our understanding of inheritance, development, and disease progression. By revealing the epigenetic mechanisms behind many diseases, epigenetic research shows the potential for targeting these mechanisms with new treatments, epigenetic drugs.

In the 2000s, the first FDA-approved epigenetic drugs appeared, and they have been raising hopes ever since. (For example, back in 2006, *Time* magazine published a cover story entitled, "Why your DNA isn't your destiny.") Epigenetics now sits squarely at the forefront of novel drug discovery, with promises of treatments for cancer and other diseases.

The most widely studied epigenetic mechanisms—DNA methylation, histone modification, and chromatin remodeling—are critical for gene and noncoding RNA expression. Moreover, these mechanisms are responsible for the sum of a person's epigenetic marks, that is, the person's epigenome.

To analyze large-scale genomic and epigenomic data, researchers, drug developers, and clinical scientists have been combining new kinds of assays, specialized sequencing methods, and more powerful bioinformatics technology. As a result, they have been identifying epigenomic disease modifications, leading to a new appreciation of its role in human disease. They have found that cancer is, by far, the largest area where epigenetics is implicated. They have also shown that epigenetics has a role in some neurological disorders and autoimmune diseases.

Alzheimer's Disease as an Epigenetic Disease

The translational science occurring in the Katz Lab at the Emory University School of Medicine surprises no one more than the head of the lab, David J. Katz, Ph.D. A classically trained developmental geneticist, whose interest lies in the development of the germline of the roundworm *Caenorhabditis elegans*, Dr. Katz has channeled his lab's work into the study of histone methylation. The lab examines how this mechanism influences cell fate, as well as how this mechanism can go awry.

The lab focuses largely on the first identified histone demethylase, lysine-specific demethylase 1 (LSD1). Already, the lab has shown that LSD1 is

see page 14

Unconventional model organisms, such as ants, can offer new, unexplored avenues to the study of epigenetics. At the University of Pennsylvania laboratory run by Roberto Bonasio, Ph.D., ants are valued because they arise from the same genome yet follow epigenetically determined developmenal paths that lead them to distinct castes in the ant colony. Curiously, epigenetic flexibility in *Harpegnathos saltator* workers (shown here hunting crickets) allows a degree of social mobility. For example, workers may rise to a reproductive rank. Knowing how such changes may occur helps researchers devise epigenetic interventions. Brigitte Baella

Circulating Tumor Cells beyond Counting

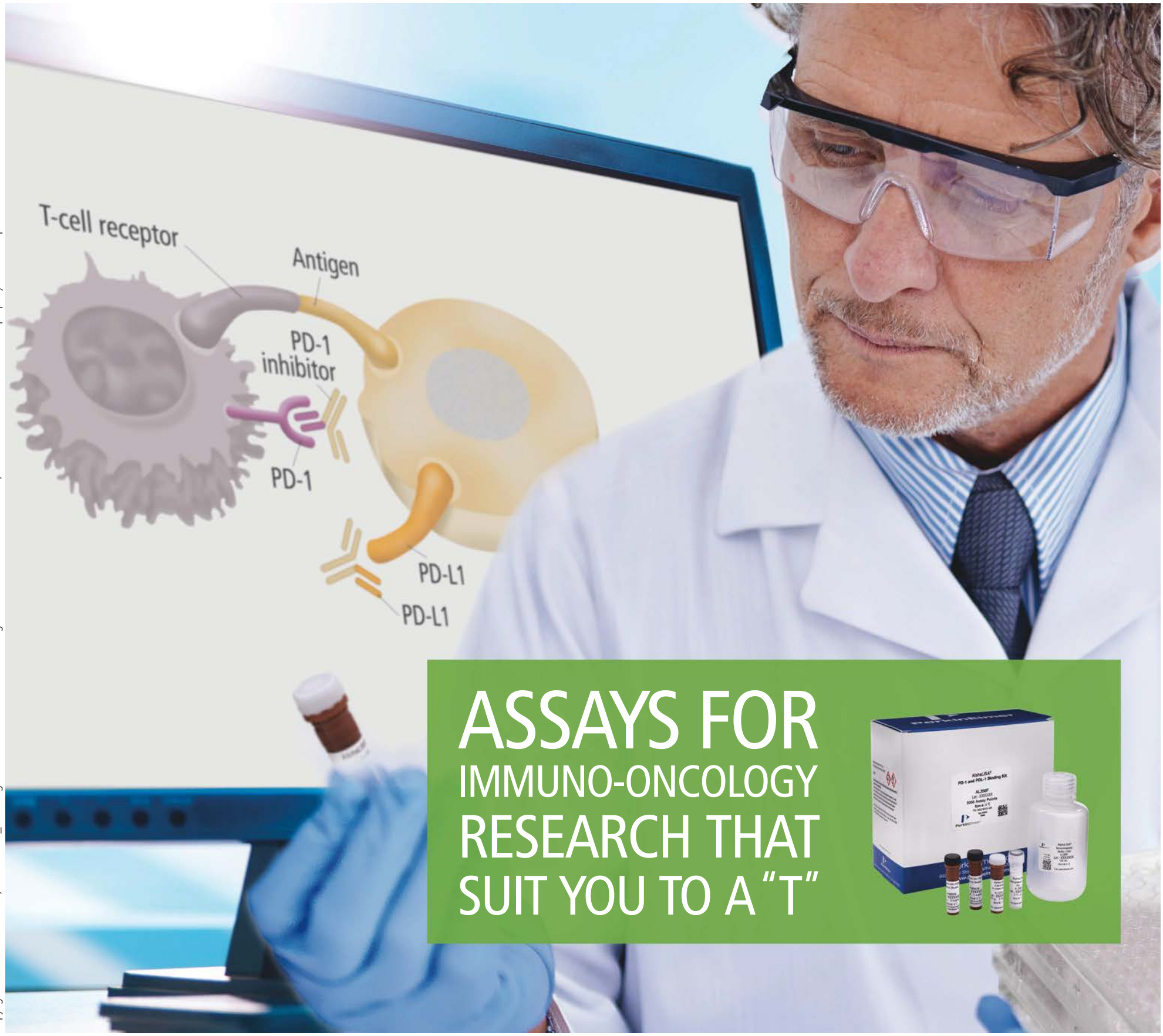
Catherine Shaffer

Slipping by unnoticed is what circulating tumor cells (CTCs) do unless they are subjected to detection, isolation, and capture technology, which is currently wrangling with the task of enumeration, that is, the generation of CTC counts—not just grand totals (for CTCs of all kinds) but subtotals (for different kinds of CTCs).

As challenging as enumeration is proving to be, many scientists are already developing technology that does more with CTCs. Don't just count CTCs, these scientists insist, preserve them for downstream applications. Culture them. Analyze their contents systematically. Use them to advance precision cancer medicine.

To accomplish these goals, scientists are trying to treat CTCs more gently. For example, CTCs that are separated from normal cells by microfluidic devices may be spared

see page 28



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What's Trending Online

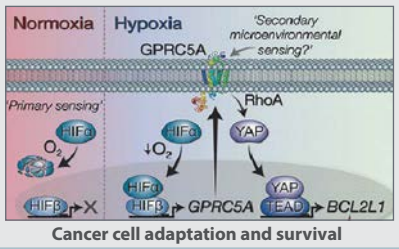
GEN NEWS HIGHLIGHTS

How Unsaturated Fats Protect against Cardiovascular Disease Revealed

Cathy Scola/Getty Images

GEN NEWS HIGHLIGHTS

Cancer's Last Gasp? Could Be, If Hypoxia Pathway Is Blocked



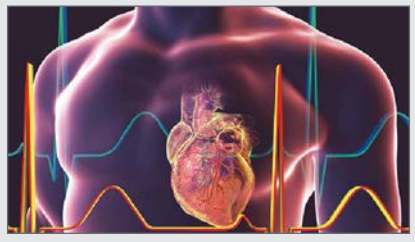
Cancer cell adaptation and survival

EMBO Molecular Medicine

Royal Veterinary College

INSIGHT & INTELLIGENCE

Duchenne Muscular Dystrophy Mutation in Dogs Reversed by CRISPR-Cas9



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GEN NEWS HIGHLIGHTS

Prescription Painkiller Boosts Risk of Heart Attack, Stroke

Alysson Muotri, UC San Diego Health

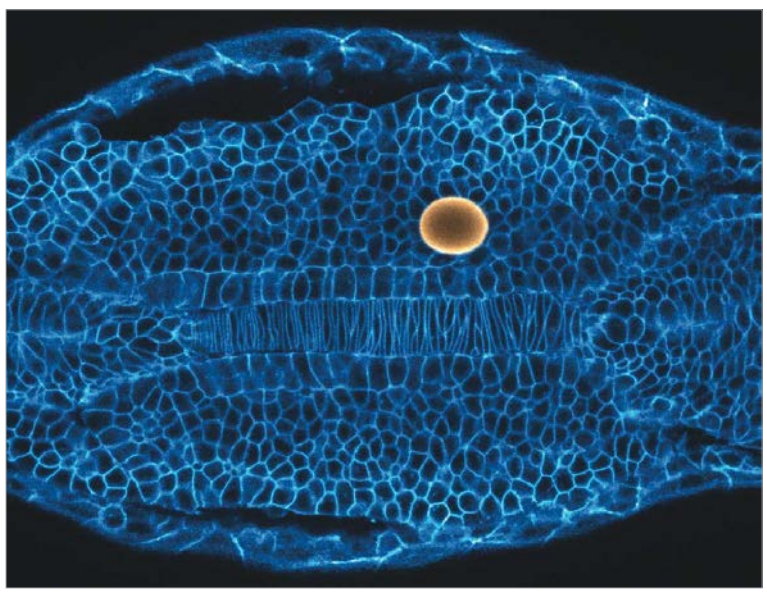
Assembly Line Mini-Brains

Researchers from UC San Diego (UCSD) have developed a new protocol for creating mini-brains. According to an article recently published in *Stem Cells and Development* (published by Mary Ann Liebert, Inc.), the new protocol can make these organoids faster and cheaper. "What we've done is establish a proof-of-principle protocol for a systematic, automated process to generate large numbers of brain organoids," said Alysson R. Muotri, Ph.D., senior author on the paper and professor in the UCSD School of Medicine. "The potential uses are vast, including creating large brain organoid repositories and the discovery of causal genetic variants to human neurological conditions associated with several mutations of unknown significance, such as autism spectrum disorder. If we want to understand the variability in human cognition, this is the first step." According to the paper, the new method would allow the simultaneous reprogramming of cortical organoids from hundreds of individuals. To accomplish this, the team compressed several steps of the process so that somatic cells are reprogrammed, expanded, and stimulated to form cortical cells at the same time. Dr. Muotri is a leader in the field, and has used the "brain-in-a-dish" approach to research Zika virus, Aicardi-Goutieres Syndrome (AGS), and to create a Neanderthalized mini-brain.

Glass Blowing, Clay Modeling, Tissue Sculpting

On Growth and Form, a book written a century ago by Scottish biologist D'Arcy Thompson, expressed a very definite idea about morphogenesis: Tissue formation is guided not just by evolution, but by physical laws and mechanics. Thompson was more right than even he might have guessed, say scientists based at UC Santa Barbara. They discovered that cells use a physical mechanism—jamming—to mold embryonic tissues into their functional 3D shapes.

Jamming occurs when particles in disordered systems, such as foams, emulsions, or glasses, are forced together or cooled down. Using magnetic droplets to probe jellyfish embryos, the scientists determined that cells exert forces on each other to alternate between liquid-like and solid-like states and form elongated structures, acting rather like the constituent materials of glass or clay sculptures. Writing in *Nature*, the scientists speculated about identifying druggable targets that could be used to manipulate jamming transitions—and give us a hand in morphogenesis.



Alessandro Mongera, University of California - Santa Barbara

Olive Oil and Sleep Keep Heart Pumping

A recent study helps explain how olive oil and other foods that are high in unsaturated fats protect against cardiovascular disease and inflammation. The answer involves Apolipoprotein A-IV (ApoA-IV), a plasma protein whose levels increase following digestion of foods high in unsaturated fats. Higher ApoA-IV levels in the blood have already been associated with lower rates of cardiovascular disease. Now, a recent study by researchers at the Keenan Research Centre for Biomedical Science (KRCBS) of St. Michael's Hospital in Toronto suggests that ApoA-IV inhibits production of platelet surface glycoproteins GPIIb/IIIa. The integrin complex, also called integrin αIIβ3, is necessary for platelet aggregation, a cause of the vessel occlusion that blocks blood flow, leading to thrombosis. The researchers showed foods with high unsaturated fats, along with appropriate sleep patterns, create the perfect combination for ApoA-IV help reduce the chances of cardiovascular disease. ApoA-IV is most active overnight and least active in the morning. "We are protected by this protein while we sleep, and most likely to experience a cardiovascular event after waking up in the morning," said Heyu Ni, M.D., Ph.D., principal investigator for the study, published in *Nature Communications*. "Mother Nature wants us to sleep well."

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the Scoop

Top 10 Companies Leveraging Gene Editing

Public and Private Companies Attract Investors, Despite Studies Raising Questions on CRISPR

Alex Philippidis

After scientists at the Wellcome Sanger Institute published findings in July that called into question the precision of CRISPR-Cas9 gene editing, three public companies focused on the technology saw their share prices fall to their 2018 lows before starting to climb back. The stock declines came despite the companies issuing statements saying either that they did not use the methods referred to in the study (CRISPR Therapeutics), or that they saw no significant impact on the development of CRISPR-based drugs (Intellia Therapeutics, Editas Medicine).

While investors in public companies both large and small may have been briefly thrown off by the negative study, and earlier studies that also linked CRISPR to failures in some circumstances, investors in private companies appear to have taken a more positive view of CRISPR and gene editing, with two companies attracting $100 million or more in investment during the first half of 2018.

The following is a list of the top 10 companies focused on gene developing and/or applying gene-editing technologies—five public companies and five private companies. The public companies are ranked by their 2017 revenues, whether from sales of products or services, or from collaborations and R&D activity. The private companies are ranked by the total capital they have raised, as disclosed in press statements. Each company is listed with a short explanation of their recent activity.

While all but one of the top public companies are developers of new treatments for disease (the other is a developer of tools), the top private companies listed offer more of a variety of specialties: Two focus on agricultural applications for gene editing, two on therapeutics, and one on tools and tech. ■

TOP **PUBLIC** COMPANIES

1 Horizon Discovery Group

2017 revenues: £36.5 million ($46.532 million)

The company expects to thrive with the RNAi and CRISPR end-markets, which are expected to grow at a CAGR of approximately 18% 2017–2021. Last year, they saw a 52% jump in revenue.

2 CRISPR Therapeutics

2017 revenues: $40.997 million

Collaborations account for all of their revenue, which jumped nearly 700% last year. But in May, the FDA imposed a clinical hold on the companies' IND for sickle cell disease candidate CTX001.

3 Sangamo Therapeutics

2017 revenues: $36.5 million

The company saw its revenue nearly double last year, rising nearly 89% over 2016, primarily due to its first collaboration with Pfizer. Recently, they announced positive preliminary data from a Phase I/II "Alta" trial.

4 Intellia Therapeutics

2017 revenues: $26.117 million

Collaborations account for all of their revenue, which jumped 58.5% from 2016, primarily due to Regeneron Pharmaceuticals licensing their CRISPR technology to develop therapies for liver diseases.

5 Editas Medicine

2017 revenues: $13.728 million

Revenue consisted of collaboration and other R&D activity, which more than doubled over 2016, rising nearly 127%. That figure reflected a decrease in expense and additional money from their partner, Allergan.

TOP **PRIVATE** COMPANIES

1 Precision BioSciences

Total capital raised: $135.65 million

The company vaulted to the top among private gene-editing companies on June 26 when it announced an oversubscribed $110 million Series B round: the third-largest VC financing among private biopharmas in Q1–2 2018.

2 Pairwise Plants

Total capital raised: $125 million

Monsanto has invested most of the $125 million raised by the company, which is an agricultural startup created to develop novel genome-editing tools leveraging the natural genetic diversity of plants.

3 Beam Therapeutics

Total capital raised: $87 million

Launched in May, the company made headlines as much for who launched the precision genetic medicine developer—its co-founders included CRISPR pioneer Feng Zhang, Ph.D.—as for what it aspires to achieve.

4 Inscripta

Total capital raised: $84.5 million

In February, they closed on $55.5 million in Series C financing. Early fruits of that labor emerged in July, when they were awarded its first U.S. patent covering systems using MAD7, the company's first free CRISPR enzyme.

5 Inari Agriculture

Total capital raised: $55 million

Recently, they added $40 million in Series B financing to their total capital raised, less than a month after the company, spun out of Flagship Pioneering, emerged from stealth mode.

Profiting from an Obsession with Technology

Enzo Life Sciences Develops, Produces, and Markets a Range of Life Science Research Reagents

Gail Dutton

After 40 years of operation, **Enzo Biochem** has a large patent portfolio. What sustains the company isn't the patents, *per se*, but a commitment on the company's part to translate the patents into useful products for customers. This focus on customers can be seen not only *within* each of the company's three subsidiaries—**Enzo Clinical Labs**, **Enzo Therapeutics**, and Enzo Life Sciences—but also *between* the subsidiaries. Given Enzo's multitiered activities, the company often can use its own products.

Enzo Life Sciences manufactures and markets biomedical research products and tools, which are critical not only to basic research, but to drug development, commercial operations, and diagnostic services.

"A lot of our technology is based on labeling molecules to see how they behave in real-world environments," Kara Cannon, Enzo Life Sciences' corporate vice president of commercial operations, tells *GEN*. "Our Proteostat® assays, for example, are used mostly by industrial bioprocessing clients.

"One pharma client was trying to understand the thermal stability of a novel therapeutic compound. Its scientists said that because they used Proteostat—which detects ligand binding without knowing the protein's function or ligand binding site, and without requiring sample separation or dilution—they were able to move their novel biomarker down the development pipeline more quickly because they were able to get results with this assay that others couldn't deliver."

The difference, Cannon explains, is sensitivity. Enzo's assay enables users to view molecular-level effects that otherwise would remain obscure.

Product Portfolio Grows Annually

Enzo's products include proteins, antibodies, peptides, small molecules, labeling probes, dyes, and kits to help researchers from pharma, biotech, and academia identify and validate targets, analyze gene expression, detect nucleic acids and protein biochemistry, and analyze cellular activity.

To enhance its reach into anatomical pathology laboratories and enable the detection of a variety of cancers, Enzo Life Sciences recently launched three new antibodies:

- $p16^{INK4A}$ monoclonal antibody, which is specific for a cyclin-dependent kinase inhibitor.
- HER2/neu monoclonal antibody, specific for a proto-oncogenic transmembrane receptor of tyrosine kinase.
- Ki-67 monoclonal antibody, which is linked to cell proliferation and therefore is a biomarker for tumor growth.

During its early years, Enzo outlicensed its technology to clients while it was building its internal infrastructure and global product portfolio. Then it added a clinical lab to provide diagnostic services to physician clients.

Hands-On Connections Remain Strong

According to Bloomberg, Enzo Biochem is an integrated diagnostic bioscience company that "engages in the research, development, manufacture, and marketing of diagnostic services and research products based on genetic engineering, biotechnology, and molecular biology."

Naturally, the company's diagnostic services fall largely within the purview of the Clinical Labs subsidiary, which includes a New York State–certified clinical lab. Research products, including those essential to the development of molecular diagnostic platforms, are available through the Life Sciences subsidiary.

"We make and sell our products, but we also use our diagnostics reagents ourselves," Cannon points out. "Our ability to validate these assays in our own labs is an asset to our customer base."

That hands-on experience dates from Enzo Biochem's first days. Some 40 years ago, Enzo Biochem's founder and CEO Elazar Rabbani, Ph.D., was a postdoctoral researcher in biochemistry at Columbia University. Back then, he was just beginning to think about forming a company.

"He was cognizant that he needed connections in the market," Cannon relates. "He tells stories of roaming the halls of Columbia, talking with colleagues." As he identified their laboratory challenges, he formed solutions and eventually turned colleagues into customers. "He built Enzo Biochem," Cannon emphasizes, "by making as many connections as possible."

At first, Enzo outlicensed its technology to clients while it was building its internal infrastructure and a global product portfolio. Then it added a clinical lab to provide full diagnostic services to physician clients.

"Throughout it all, our values stayed the same," Cannon maintains. "Enzo is publicly traded (NYSE:ENZ), but it has the feel of a positive, proactive family business. We'll roll up our sleeves to get product out the door and to our customers."

Obsessed with Technology

"We're obsessed with technology," Cannon declares. This fixation on developing and cultivating strong intellectual property, she continues, meant that some company activities, like focusing on branding, were a lower priority than helping clients build their own brand equity.

"In hindsight, we should have started branding earlier," Cannon admits. By outlicensing its products, the company kept operating in stealth mode. "Today, we're not as well-known as we'd like," she says. "We have a lot of repeat customers and about $100 million per year in revenue, but I still go to conferences where the Enzo name is new to people."

Yet, she points out, Enzo has a proud history. It was a pioneer in fluorescent labeling, launching those products in 1981. At that time, radioactive labeling was used routinely to study nucleic acids.

"That technique wasn't user friendly or safe, and it required a special license and a special room to work with the isotopes," she says. When fluorescent labeling was devel-

Circadian Rhythm Gene May Serve as Target for Glioblastoma Therapies

Scientists from the Virginia Tech Carilion Research Institute say a gene involved in the body's circadian rhythms is a potential target for therapies to help patients with glioblastoma. Their discovery ("Casein Kinase 1 Epsilon Regulates Glioblastoma Cell Survival"), published in *Scientific Reports*, points to a subtype of a particular gene that apparently is enabling the survival of cancer cells, although it is more commonly associated with circadian rhythms.

"The world is desperately seeking new treatments for glioblastoma and no one has ever before pointed to this gene as a target upon which to base therapies," said Zhi Sheng, Ph.D., an assistant professor at the Virginia Tech Carilion Research Institute, whose team pinpointed the gene from 20 suspects it had previously identified. "More research is needed before a treatment can be designed, but our early, basic science results are promising.

"We have found that inhibiting this gene [casein kinase 1 epsilon] may inhibit cancer stem cells from renewing themselves and differentiating into glioblastoma cells, which we suspect may be a hallmark of this very persistent cancer," explained Dr. Sheng, who is also an assistant professor of Internal Medicine at the Virginia Tech Carilion School of Medicine.

Dr. Sheng and colleagues, also evaluated two commercially available drugs that block the casein kinase 1 gene from activating circadian rhythms. ■

oped, the work became more efficient, less expensive, and safer."

Three years later, in 1984, Enzo launched *in situ* hybridization (ISH) probes and FISH (fluorescent *in situ* hybridization) technology.

Since then, Enzo has grown by releasing new products each year and by acquiring strategic companies. For example, Enzo Life Sciences acquired **Alexis Biochemicals** and **Axxora** in 2007, **Biomol International** in 2008, and **Assay Designs** and **Stressgen Biotechnologies** in 2009.

Meeting Today's Challenges

"With virtually all of our customers, from academic researchers to pharma, biotech, and clinical labs, cost pressures continue to be the number one concern," Cannon insists. To reduce the pressure, she says Enzo Life Sciences works with customers to help ensure that its reagents fit into labs' existing workflows. "There's no need to buy capital equipment," she stresses.

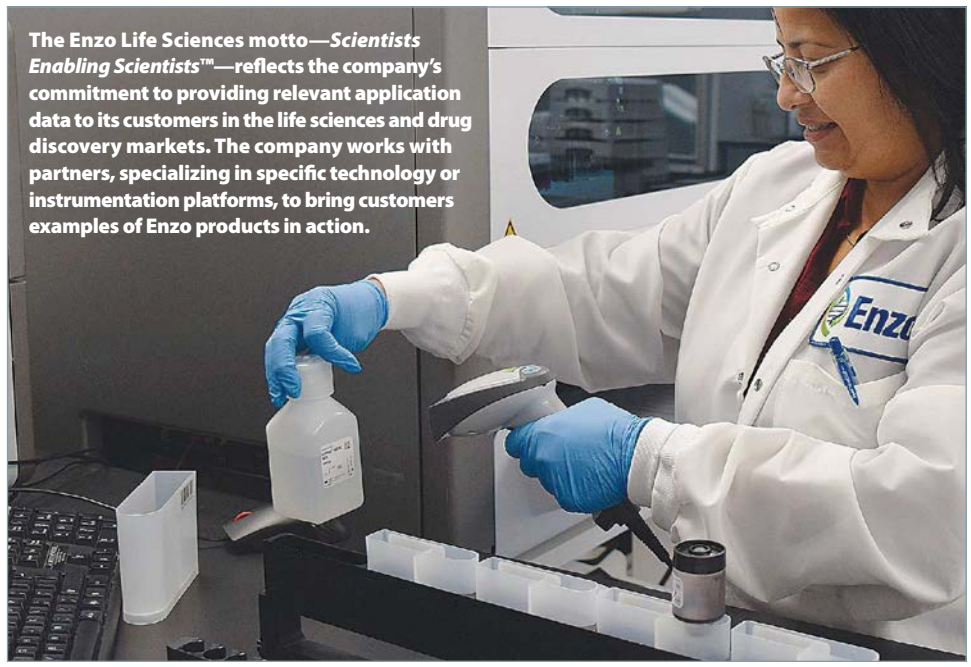
The company focuses on offering open, flexible solutions that allow more cost-effective options, adds Cannon. It also recognizes the importance of steadily incorporating technological advances, particularly those that can help clients achieve results more quickly, whether the clients are advancing their products toward clinical trials or scaling up commercial operations.

"We're continuing to expand our already broad base of technologies," states Cannon. "We're looking to move the Proteostat assay family to areas outside its current utility, like flow cytometry, and to find ways to put technology into alternate formats."

In 2018, the company has secured 14 new patents and added 6 new products (so far). It has become the lab services provider to a large commercial payer and has expanded its sales force. Finally, the firm has taken steps to intensify its partnering activity. Also, according to some analysts, Enzo is planning to expand its facilities.

To help Enzo stay abreast of current and future research interests, the Enzo commercial team is engaged in the field, talking with customers, and staying active at trade shows and on social media, according to Cannon, who adds that the company also combs the literature to identify new and emerging areas of research, gathering information to support the development of assays that can serve up-and-coming sectors.

Like Dr. Rabbani in Enzo's earliest days, Cannon says, "We try to stay as connected as possible to our customers, to really hear what they're saying." GEN



The Enzo Life Sciences motto—*Scientists Enabling Scientists*™—reflects the company's commitment to providing relevant application data to its customers in the life sciences and drug discovery markets. The company works with partners, specializing in specific technology or instrumentation platforms, to bring customers examples of Enzo products in action.

Vital Signs

Enzo Life Sciences

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Principal
Elazar Rabbani, Ph.D.
Chair and CEO

Number of Employees
475

Focus
Enzo Life Sciences offers a portfolio of proteins, antibodies, peptides, small molecules, labeling probes, dyes, and kits. The company provides tools for target identification/validation, high-content analysis, gene expression analysis, nucleic acid detection, protein biochemistry detection, and cellular analysis.

When Success Rates Go Low, Cell-Based Screens Go High

Richard A. Stein, M.D., Ph.D.

Over the past 10 years, the costs of developing a new therapeutic drug, from research and development to marketing approval, has more than doubled. Yet only about 1 in 1000 compounds progresses from preclinical studies to human testing. Many compounds identified from large screens fail during clinical trials, typically during Phase II, motivating drug developers to reconsider their screening strategies.

For example, many developers are looking for ways to implement high-content screening while maintaining acceptably high levels of throughput.

"If we look at drug discovery over the past three decades, one conclusion, for both small molecules and proteins, is that we have been overly simplistic in the way we identify early drug candidates," says Jean-Philippe Stephan, Ph.D., director of the Center of Excellence for Pharmacological Screening, Compound Management and Biobanking, Institut de Recherches Servier. A key effort in Dr. Stephan's group focuses on identifying, early during the research phase, chemical compounds that have the potential to be developed into therapeutic agents.

"As part of this work, we made investments in platforms to perform high-content screening," reveals Dr. Stephan. Drug discovery efforts at Servier focus on therapeutic areas that include oncology, metabolism, immuno-inflammation, neurobiology, and cardiovascular diseases.

Balancing Content and Throughput

"For a long time, we thought that drug discovery was just a matter of numbers, and that if we looked for a needle in a haystack and screened as many compounds as possible, we would end up finding that needle—even if we used a simple screening method," relates Dr. Stephan. This view encouraged the development of large chemical libraries, containing millions of compounds, that could be interrogated to identify drugs of interest.

"But when screening millions of compounds, one cannot be too complex," cautions Dr. Stephan. "Screening has to be done fast because drug development is a race."

In a recent study that discussed the sustainability of current drug discovery approaches, Dr. Stephan and colleagues highlighted the key benefits and challenges of high-content analysis for phenotypic drug discovery. The study considered how high-content capabilities, such as image capture and processing and data analysis, could be incorporated into large-scale discovery efforts while sustaining sufficient throughput.

"We need a model that in some way recapitulates what is going on in the body," insists Dr. Stephan. "But that is very difficult, and even more so in high-throughput or high-content screening."

For example, for decades, cells have been grown using two-dimensional culture systems, which do not reproduce the physiology and the elasticity of human tissue. Three-dimensional culture systems model the physiology of live tissues more accurately, but their use is still at relatively early stages.

"To reproduce physiological conditions, one has to mix different types of cells," says Dr. Stephan. "The main limitation is how to reproduce the complexity of the human body in a well."

Even when an ideal experimental model is in place, developers must deal with another difficulty: selecting the best readout. Examples of readouts include the size of the nucleus, the intensity of a specific stain, the presence of an antibody in a specific cellular compartment, or the movement of the cell.

One of the strengths of high-content screening is the possibility of measuring multiple parameters at the same time. However, virtually all readout types face technical limitations. For example, the number of wavelengths that can be resolved by a microscope is normally limited to four. The limited number of choices for some readouts can bias the biological question that is being explored, and an in-depth knowledge of the biology is therefore critical to allow the selection of the best readout.

Yet another difficulty is the need to limit the potential for data bias, such as that which arises when positive controls are used. In high-throughput screening (HTS), positive controls are needed at multiple steps, such as assessment of plate quality, or optimization of experimental design. Historically, controls have been regarded as little more than a technical issue, but developers are starting to appreciate the essential roles of several types of controls at multiple steps of the discovery process.

Finally, high-content screening needs to continue incorporating machine learning algorithms, which are expected to become routine in the earliest steps of drug discovery. "Artificial intelligence will enable us to integrate a huge mass of information," predicts Dr. Stephan.

He points out, however, that information that is accumulated or examined piecemeal may be inaccurate or capture too little biological complexity. Comprehensive approaches, in contrast, would be more powerful. They could even streamline screening efforts.

Deep neural networks could help preselect some com-

Researchers agree that high-content screening needs to continue incorporating machine learning algorithms, which are expected to become routine in the earliest stages of drug discovery.

Andrew Brookes/Getty Images

pounds in a first phase of the screening. "Then we can go into an enriched set of compounds," explains Dr. Stephan, "and use a more complex model to rapidly identify compounds that have a chance to be successful."

Spheroid Optical Clearing

"We developed a high-throughput pipeline for spheroid optical clearing, fluorescent high-content confocal imaging, and nuclear segmentation," says Molly E. Boutin, Ph.D., biologist at the National Institute of Health's National Center for Advancing Translational Sciences. This work is helping to refine 3D cell culture models, which are already contributing to drug discovery.

Many of the 3D models that have been used to interrogate biology have been conducted with spheroids. In spheroids, however, tasks such as imaging and data analysis still pose challenges in HTS.

"Imaging through layers of cells generates a lot of light scattering," points out Dr. Boutin. Optical clearing protocols were developed to allow imaging deeper in tissues, but only a limited number of studies have explored them in a high-throughput context. "These are very simple analysis techniques," she continues. "They usually don't look at where the cells are three dimensionally within the spheroid."

For example, to predict the cytotoxicity of a drug, the size of a sphere in a bright field microscopy image would be used as an indicator of cell death. "But that does not indicate whether the cells that are dying are on the outside or on the inside of the sphere, or what exactly is going on," says Dr. Boutin.

Dr. Boutin and colleagues recently developed a high-throughput spheroid optical clearing and nuclear segmentation pipeline and described how it was used to examine about 558,000 image files from 3000 spheroids derived from breast carcinoma and primary glioblastoma cell lines. Using this automated protocol, the scientists could image a 384-well plate in 1–2.5 hours. Also, the platform allowed the scientists to customize post-segmentation analyses based on individual users' needs. In this proof-of-concept study, Dr. Boutin and colleagues demonstrated the ability of the segmentation algorithm to identify several subpopulations of fluorescently labeled cells within individual spheroids.

"One of the fields that is growing at present is that of machine learning algorithms, which allow users to train a program to learn what a dataset is like," informs Dr. Boutin. "From the learning process, one can predict what an unknown dataset would be."

For example, using a control and treatment image dataset, one may train the program to determine whether an unknown treatment would cause a specific phenotype. An advantage of machine learning is the introduction of less user bias for steps where a threshold is manually chosen. "We did not include any machine learning analyses in our algorithm," admits Dr. Boutin, "but there are a few parts of the analysis where we would like to do that."

Directed Differentiation

"Our lab is interested in how different genetic and environmental factors contribute to disease progression and how we can find drugs that can rescue the defects," says Shuibing Chen, Ph.D., associate professor of surgery and biochemistry, Weill Cornell Medical College. In a recent study, Dr. Chen and colleagues developed a differentiation protocol to examine the role of *Glis3*, a gene associated with type 1 and type 2 diabetes, in the biology of human pancreatic beta cells.

This work revealed that the loss of *Glis3* from human embryonic stem cells impaired their differentiation into pancreatic progenitor and β-like cells and increased the death of these cell types. As part of a high-content chemical screen that looked for candidates that could rescue this increased cell death, investigators in Dr. Chen's lab identified a TGF-β inhibitor that is currently in Phase II trials.

The TGF-β inhibitor, galunisertib, specifically rescued the cell death caused by the *Glis3* deletion *in vitro* and *in vivo*, without affecting wild-type cells. The high-content approach used to identify galunisertib as a drug candidate could be applied more generally. "High-content screening," Dr. Chen predicts, "will be more and more broadly used in drug screening."

The protocol developed by Dr. Chen and colleagues for differentiating individual pancreatic cell types and modeling human disease has several advantages. "When we perform screenings, we can combine insulin, which is a β-cell marker, and glucagon, which is an α-cell marker, and we can obtain information about small molecules that promote differentiation to either of these cell lineages," explains Dr. Chen.

Another advantage is the ability to evaluate cell death and cell proliferation at the same time. Interrogating cell death and cell proliferation markers together could lead to the identification of certain small molecules that cause cell death, and others that block cell proliferation, as opposed to causing cell death. "In that scenario," asserts Dr. Chen, "we already have some mechanistic clues

See Success Rates on page 10

Success Rates

Continued from page 9

from the primary screening of the cells."

Flow Cytometry in Antibody Discovery

"We developed an approach that relies on high-throughput flow cytometry (HTFC) to identify antibody binders," says Yana Wang, Ph.D., scientist, lead discovery, Oncology Discovery Unit, **Takeda Pharmaceuticals**. To support antibody discovery programs, Dr. Wang and colleagues at Takeda developed a HTFC approach that combines the iQue Screener with a modular robotic system. The approach amounts to a HTFC workflow.

By incorporating sample miniaturization, acquisition speed, and plate-based data management, HTFC provides a flexible and modular solution for integrated applications, and it allows multiple parameters to be concomitantly measured robustly and accurately. HTFC enabled Dr. Wang and colleagues to multiplex cytokine beads and several cell types.

Essentially, the scientists used HTFC to demonstrate how multiple cytokine levels and cell activation parameters could be monitored simultaneously, providing high-content information. The scientists also showed how their new platform could be more efficient than older platforms, which use 96-well plates and require manual sample preparation. The new platform allowed sixteen 384-well plates to be processed within 8 hours.

In previous years, Takeda scientists established powerful programs for the HTS of small-molecule therapeutics. "We are trying to use this already established high-throughput screening infrastructure to develop platforms that support the needs of Takeda's growing biologicals programs," says Dr. Wang.

While some of the challenges are shared between the two types of therapeutics, biologicals present an additional set of unique limitations. As indicated by Dr. Wang, "Normalizing all the purified antibodies to the same starting concentration, developing the best storage conditions, and optimizing buffers—these are some of the challenges that we are specifically addressing for biologicals." GEN

ADHD Doubles Risk of Early Onset Parkinson's

University of Utah (U of U) Health researchers released some interesting, if not disturbing new findings: attention-deficit hyperactivity disorder (ADHD) patients have a greater risk of developing Parkinson's and Parkinson-like diseases than individuals with no ADHD history. Their study appears in *Neuropsychopharmacology* through an article titled "Increased risk of diseases of the basal ganglia and cerebellum in patients with a history of attention-deficit/hyperactivity disorder."

This new data is significant as 11% of children (4–17 years old) nationwide have been diagnosed with ADHD. Moreover, the long-term health effects of having ADHD and of common ADHD medications remains understudied.

"Parkinson's disease is commonly thought of as a neurodegenerative disease associated with aging," explains senior study investigator Glen Hanson, D.D.S., Ph.D., professor of pharmacology and toxicology in the School of Dentistry at U of U Health. "This may be the first time where a childhood disease and its treatment may be linked to a geriatric expression of a neurodegenerative disorder."

Employing a retrospective, population-based study, the research team found that ADHD patients were more than twice as likely to develop early onset (21–66 years old) Parkinson's and Parkinson-like diseases compared to non-ADHD individuals of the same gender and age. Amazingly, the estimated risk was six to eight times higher for ADHD patients prescribed stimulant medications, including methylphenidate (Ritalin, Concerta, Daytrana, Metadate, and Methylin), mixed amphetamine salts (Adderall), and dexmethylphenidate (Focalin). ■

How Does BRET Indicate Trafficking of Endogenously Expressed Receptors?

Combined Progress: CRISPR-Cas9 Enables Endogenous Donor Expression and CLARIOstar Sensitive Detection

Andrea Krumm, Ph.D., Applications Specialist, BMG LABTECH

G-protein-coupled receptors (GPCRs) are estimated to be targeted by 35% of FDA-approved drugs and constitute the biggest protein group targeted by drugs.[1] Not surprisingly, pharmaceutical research extensively studies GPCRs which transmit signals from the outside to the inside of a cell. It is not only of interest whether a compound binds a specific receptor to activate or inhibit it, but also which signaling is triggered and whether any further receptor regulation follows an initial binding event.

A popular tool to measure receptor binding is bioluminescence resonance energy transfer (BRET) in microplate format. The energy transfer takes place between the light emitted by a luciferase and a fluorophore only if both are in highest proximity. Typically, the receptor of interest is labelled with a luciferase that emits light when converting its substrate. Expression of the fusion protein is achieved by stably or transiently transfecting it into a cell line of interest leading to exogenous and, hence, non-physiologic receptor levels. The luciferase-labelled receptor acts as a donor for a fluorophore which is fused to an interaction partner. Only if that is bound to the receptor, the fluorophore accepts the energy coming from the receptor-bound luciferase and emits light. The ratio of emission light from acceptor fluorophore to luciferase signal reveals the interaction of both.

So far, the method was limited by exogenous donor expression and by the use of a single acceptor, limiting the analysis to one interaction. Following, it is explained how CRISPR-Cas9 gene editing was used to express the donor GPCR at endogenous level and how those cells assisted in monitoring receptor trafficking by using a dual-acceptor approach.

Endogenous Receptor Expression Is Sufficient for Monitoring Protein Interaction

The chemokine receptor type 4 (CXCR4) is a GPCR implicated in cancer progression and therefore is a target for cancer therapy.[2] Upon binding its ligand CXCL12, CXCR4 is activated and then recruits β-arrestin2 (β-arr2). Subsequently, the receptor is internalized for either recycling back to the plasma membrane or lysosomal breakdown. A group of researchers from the Australian Harry Perkins Institute of Medical Research was interested in studying these mechanisms of CXCR4.

To this end, the group used CRISPR-Cas9 to insert the DNA coding for nanoluciferase (Nluc) into the endogenous locus of CXCR4 in HEK293 cells.[3] This way, the resulting BRET donor (receptor + luciferase) was expressed at endogenous level. The Nluc was chosen as it is very bright and results in sufficient light emission despite low expression levels.

In an initial experiment, the CRISPR-Cas9–edited cells were tested regarding their suitability for BRET experiments. The CXCR4/Nluc expressing cells were transiently transfected with cDNA coding for a β-arr2/Venus BRET acceptor *(Figure A)*. As soon as cells expressing BRET-donor and -acceptor were treated with CXCL12, the CRXCR4 ligand, the BRET ratio increased and reached its maximum approximately 90 s after treatment *(Figure B)*. This is explained by the recruitment of β-arr2/Venus to CXCR4/Nluc, a well-established step in receptor-regulation. The resulting proximity of Nluc and Venus fluorophore allows energy transfer and increased emission of Venus and consequently a higher BRET ratio.

Dual BRET Receptor Approach Reports on Receptor Trafficking

After demonstrating that the BRET assay with endogenous levels of the donor works, the researchers went one step further: they asked whether it is possible to combine the CXCR4/Nluc BRET donor with two acceptor fluorophores exhibiting different spectral properties and different intracellular interactions sites. They hoped to enable the NanoBRET™ method to monitor receptor trafficking. The interaction partners the group chose were K-ras as a membrane marker and Rab4 as a marker for the early endosome where the receptor is prepared for recycling. K-ras was fused to the NanoBRET 618 acceptor emitting in the red range and Rab4 to Venus emitting in the green range. Both acceptors were transiently transfected into the CRISPR-Cas9 genome-edited cells expressing CXCR4/Nluc *(Figure C)*.

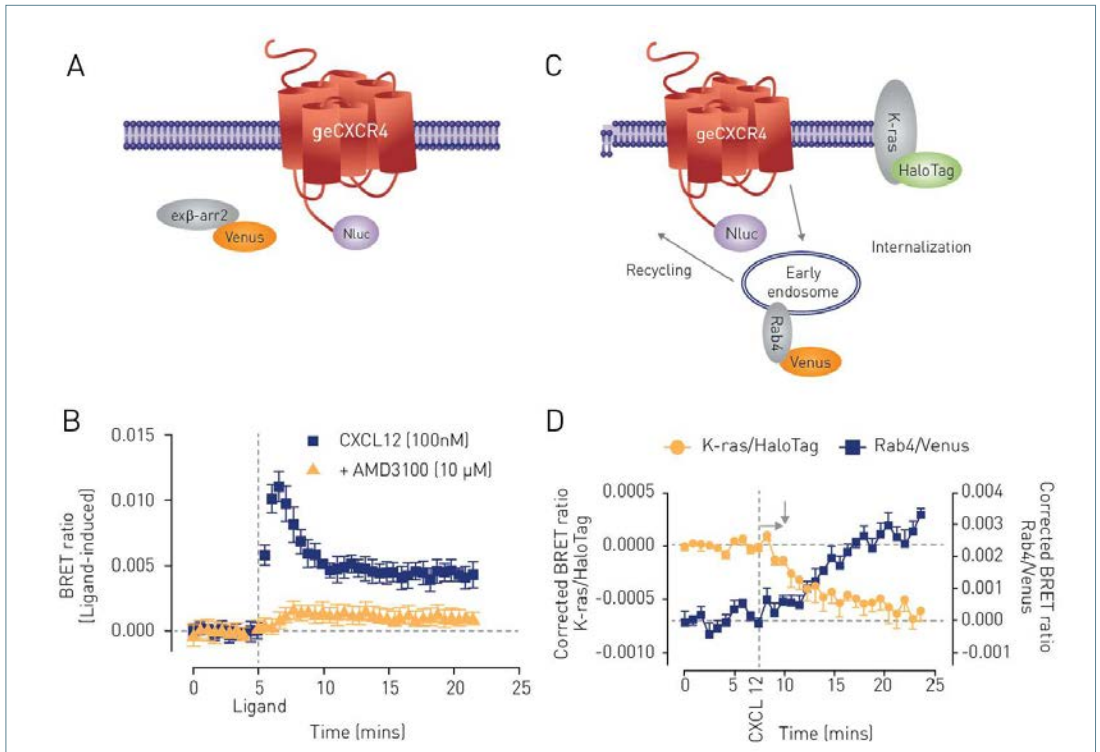

Figure. Assay principles (A, C) and results (B, D) of novel NanoBRET approaches. A and B show BRET between endogenously expressed CXCR4 and β-arrestin2. C and D show novel dual acceptor approach indicating intracellular receptor trafficking.

From the analysis point of view, the dual acceptor approach is particularly challenging. First of all, emissions from three light-emitting molecules need to be separated: blue signal coming from the Nluc (donor), red signal (membrane marker, KRAS/NanoBRET 618), and green signal (early endosome marker, Ra B4/Venus).

Microplate readers usually measure BRET with filter-based systems consisting of two filters: one for the luciferase and one for the acceptor fluorophore. This is clearly no option for the Australian researchers. Monochromators offer the possibility to measure at any and several wavelengths. However, they are mostly limited in sensitivity. As the dual acceptor BRET works with endogenous and low levels of the donor, a low signal is expected and the detection needs to be extremely sensitive.

The group found a solution in the CLARIOstar® microplate reader with a patented linear variable filter (LVF) monochromator. The unique monochromator uses variable filters for wavelength selection, which allows it to use broad bandwidths and provides better light transmission, both leading to the highest sensitivity of monochromator-based readers.

Using the CLARIOstar for detection of the dual acceptor BRET assay has proven successful: Cells expressing CXCR4/Nluc at endogenous level and the two acceptors, K-ras/Nano BRET 618 and Rab4/Venus, responded to treatment with the ligand CXCL12. The BRET ratio for the membrane marker K-ras decreased upon treatment, indicating dissociation from the membrane. In contrast, BRET ratio of the endosome marker Rab4 increased, which reports the shuttling of CXCR4 to the early endosome upon agonist treatment *(Figure D)*.

CRISPR-Cas9 and State-of-the-Art Detection Technology Expand NanoBRET Technology

The novel CRISPR-Cas9 technique successfully fused the Nluc BRET donor to endogenously expressed CXCR4. Luminescence generated by the resulting protein-luciferase fusion was sufficient to monitor receptor-protein interactions as well as trafficking. The multiplex internalization assay depends on two acceptor fluorophores whose selective detection was rendered possible by the CLARIOstar's monochromator. GEN

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High-Quality Hits from High-Throughput Screens

Optibrium Created a Multiparameter Approach to Identify Good SAR, Potent Compounds

Tamsin E. Mansley, Ph.D., Peter A. Hunt, Ph.D., Edmund J. Champness, and Matthew D. Segall, Ph.D.

High-throughput screening (HTS) campaigns are frequently carried out early in a drug discovery project for target validation and identification of validated hits. In HTS analysis, it is important to:

- Quickly identify one or more hit series with high activity. Diversity between series is beneficial to provide backup strategies.
- Ensure lead series exhibit structure-activity relationships (SARs) that indicate opportunities for further optimization.
- Find compounds with good lead-like properties that provide high-quality starting points for hit-to-lead exploration, these include:
  - ❍ Appropriate physicochemical properties.
  - ❍ Good absorption, distribution, metabolism, and excretion (ADME) properties.
  - ❍ Avoiding frequent hitters (false positives) and high-risk functionalities.

To identify such compounds and series, a common practice is to apply filters to the typically large HTS datasets, for example, by specifying an activity threshold or simple properties such as molecular weight, lipophilicity, or the presence of substructures that may indicate nonspecific binding. This practice, however, draws artificially harsh distinctions between compounds, given the inherent variability in HTS data and the low correlation between simple properties and the ultimate *in vivo* disposition of a compound.

Consequently, the common practice can lead to the selection of false positives (that is, active compounds that are not good starting points for further optimization) and rejection of false negatives (that is, potentially good compounds that have been inappropriately rejected). Alternatively, using a rigorous multiparameter approach enables appropriate weight to be given to these data to confidently identify high-quality, potent hits while avoiding missed opportunities.

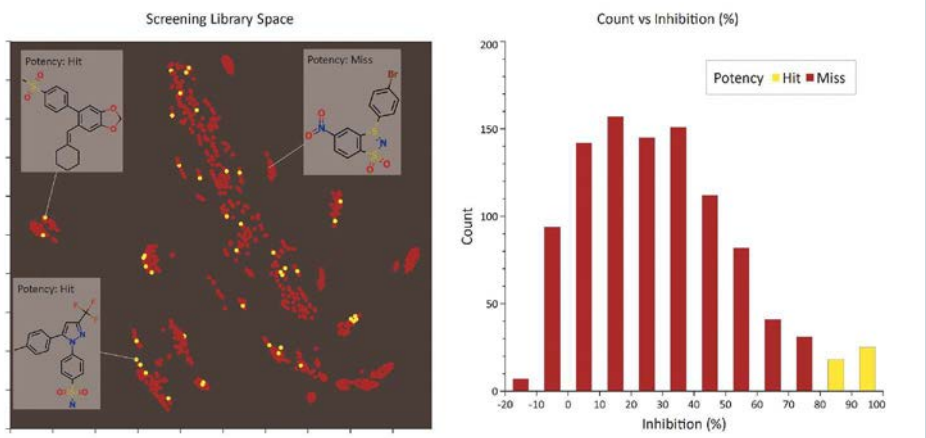

Figure 1. Chemical space of a 1000-member screening library. Some areas of chemistry possess no active compounds, whereas in other regions, clusters or hot spots of activity can be observed. A "hit" for this data set is defined by inhibition values >80%, where the mean is 31% and the standard deviation is 24%.

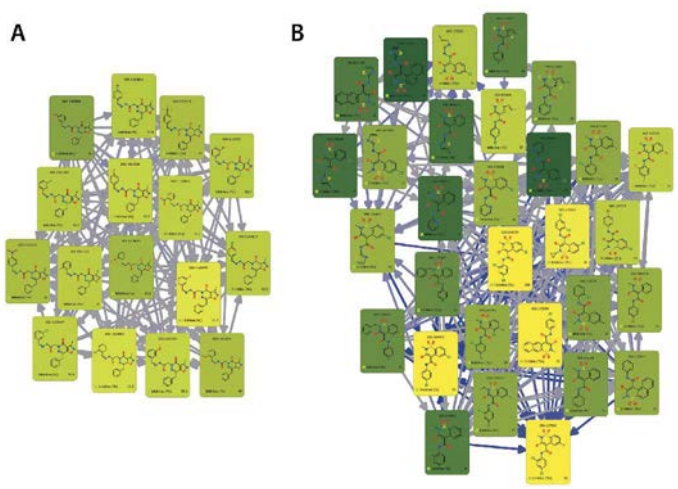

Figure 2. Extract from an activity landscape visualization. Layout (A) is an example of a "flat spot"; layout (B) illustrates a variable region.

Mapping the Chemical Space of Activity

The structural diversity of the compounds screened during a campaign can be explored by clustering or visualization of a chemical space, in which each point represents a single compound, and similar compounds are clustered together. Mapping compound activities onto the visualization using color makes it easy to see hotspots with multiple highly active compounds that might be interesting for further investigation.

One of the first steps in an HTS analysis is to determine, "What is a significant result?" One approach is to assess the distribution of the activity across the dataset and select those compounds with significantly higher activity than average. For example, a compound might reasonably be classified as a "hit" (*Figure 1*) if its activity is more than two standard deviations above the mean.

Understanding the Activity Landscape

Another goal in the analysis of HTS data is to identify a series containing potent compounds with good SARs—this provides confidence that hits are genuine and not the result of assay interference or impurities. Consistent SARs may also indicate opportunities for further optimization.

Analyses, such as the activity landscapes displayed by StarDrop™ software solutions, quickly highlight interesting regions of SARs (*Figure 2*). This method highlights the difference in potency between every pair of similar compounds in a set:

- Variable regions highlight large changes in activity resulting from small changes in structure and indicate interesting SARs.
- "Flat spots" indicate limited opportunity for optimization of activity. These highlight opportunities to optimize different properties without a negative impact on activity.

Targeting High-Quality Hit Series

While drug candidates should have a SAR consistent with potency, they are more likely to be successful if they also balance appropriate selectivity, ADME, and physicochemical properties. Considering these properties as early as possible, using multiparameter optimization (MPO), is important when assessing a potential hit or lead series. A high-quality lead compound may demonstrate a combination of:

- Low molecular weight, offering more flexibility for structural optimization.
- Low lipophilicity, reducing the risk of off-target effects and providing a better chance of good solubility/permeability.
- Appropriate ADME properties, tailored to the project's therapeutic objectives.
- An absence of undesirable structural features, for example, those found in pan-assay interference compounds (PAINS),[1] as these may be promiscuous binders, resulting in false positives.

A common approach to selecting compounds based on multiple properties is to apply a series of filters to the data, a simple example of which is shown in *Figure 3A*. However, this illustrates that while there are numerous compounds in the 1000-member screening library that might be considered as hits based on the inhibition data, only 4 of these also pass filters for 3 additional simple lead-like properties.

Any filtering approach risks inappropriately excluding potentially good compounds by ignoring the relative importance of each criterion and uncertainty in the data. Uncertainty can come from a variety of sources:

- Experimental variability in the assay, assessed by the variability observed for reference compounds or the standard error in the mean of replicate measurements.
- Statistical uncertainty for *in silico* predictions.
- The relevance of the property to the outcome of the project. For example, many compounds with PAINS alerts are not frequent hitters, and several successful drugs contain PAINS alerts.[2]

A more appropriate method for MPO will take into consideration the uncertainty in the data and weight the input data based upon their relevance. An example of this is Probabilistic Scoring[3] in StarDrop, where compounds are scored based upon the likelihood of their meeting the criteria in a scoring profile, as defined by the project's objectives. In this approach, desirability functions can be specified for each property, rather than employing hard cut-offs.

In addition, this method explicitly takes into consideration any uncertainty in the data, to avoid rejecting compounds inappropriately where the data do not confidently determine their outcome against the criteria. This results in MPO scores with known uncertainties, and this enables us to consider

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when compounds can be selected or rejected with confidence.

Figures 3B & 3C illustrate the Probabilistic Scoring approach. The Simple Profile strategy (*Figure 3B*) considers the same properties as the filtering strategy (*Figure 3A*) but applies desirability functions weighted by the property's importance. The selection (yellow) includes all the high-scoring compounds that cannot be statistically differentiated (compounds where the error bars overlap with those of the top-scoring compound, as shown by the inset "snake" plot).

Sampling on this basis highlights a much more diverse sample across chemical space, identifying several interesting compounds that would otherwise have been missed by filtering. The balanced profile in *Figure 3C* includes ADME properties desirable for a high-quality lead, in addition to potency and physicochemical properties. While this selection also samples broadly across the chemical space, some additional series are excluded, and we could have even greater confidence that the remaining compounds have a good balance of properties.

Conclusions

Several factors need consideration when prioritizing compounds and series from an HTS campaign. High-quality compounds will exhibit potency combined with a balance of lead-like physicochemical and ADME properties, making them amenable to hit-to-lead optimization. Demonstration of SARs within a series provides opportunities and directions for further optimization.

Assessing the quality of hit compounds using filters risks excluding potentially interesting compounds due to the inherent uncertainty in the data available during drug discovery. Instead, employing a rigorous approach to MPO can ensure that any uncertainties in the data are taken into consideration, enabling the identification of high-quality, potent compounds quickly. From this, one can confidently select structurally diverse series for further exploration and avoid missed opportunities from any HTS campaign. GEN

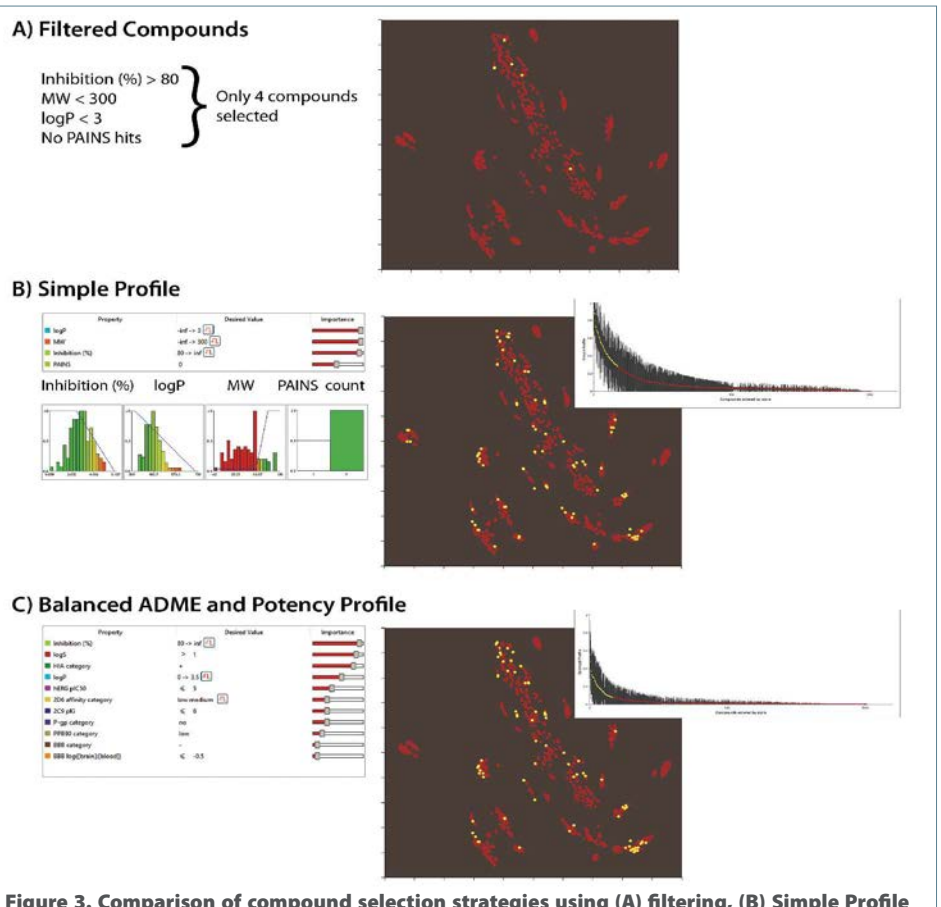

Figure 3. Comparison of compound selection strategies using (A) filtering, (B) Simple Profile including desirability functions and relative importance, and (C) MPO scoring using a balanced project profile. Compound selections (yellow) include those compounds with scores that cannot be differentiated from the highest-scoring compound with statistical confidence.

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Epigenetics Revitalizes Drug Discovery

Continued from page 1

required in the germline to reprogram histone H3K4 methylation. In addition, the lab has shown how this pathway prevents epigenetic transcriptional memory from being inherited transgenerationally.

When Dr. Katz and colleagues moved their work into a mouse model, they inadvertently discovered massive neurodegeneration and paralysis when LSD1 was eliminated from adult mice. One experiment resulted in their mice having massive neurodegeneration and paralysis, leading to the model that LSD1 plays a role in Alzheimer's disease (AD) and a related condition, frontotemporal dementia. Indeed, when an LSD1 antibody was applied to postmortem AD patient's brains, it colocalized with tau protein aggregates—the hallmark of AD. This was a remarkable result as very few proteins have been shown to colocalize with aggregates.

Dr. Katz anticipates that his lab's work will advance drug discovery through epigenetics. If no other company exploits the work, his will, even though it currently exists only on paper.

"Most of the epigenetic drugs are cancer therapies," he notes. "More recently, drugs that target epigenetic enzymes have been pursued for a wide range of diseases, ranging from muscular dystrophies to Alzheimer's disease. Going forward, it will be exciting to see if epigenetic-based therapies prove to be effective against other diseases."

Key Epigenetic Findings from Ants

Ants are a powerful model to study epigenetics. Why? Because different types of ants in the colony arise from the same genome. "Workers and queens have the same genes," points out Roberto Bonasio, Ph.D., an assistant professor of cell and developmental biology at University of Pennsylvania's Perelman School of Medicine. "Among other things," he tells *GEN*, "this means that the same genome specifies at least two types of brain that behave in dramatically different ways." If we study how epigenetics influences the brains of ants, we might gain insights of general significance.

Dr. Bonasio started his postdoc intending to use biochemical methods to study gene regulation, but the rise of new technology changed his plans. "The deep sequencing revolution came," he recalls, "so everything went from single locus and in vitro systems to genome-wide *in vivo*." Now it is hard, he continues, "to find a chromatin and epigenetics paper without lots of genomics and bioinformatics."

He is particularly excited by new work suggesting that noncoding RNAs in the nucleus influence transgenerational epigenetic inheritance. This work, which is highly controversial, challenges the longstanding notion that most if not all epigenetic marks are erased in the germline (at least in mammals), such that every new individual starts with a clean genomic slate.

To study whether noncoding RNAs carry epigenetic information across generations, Dr. Bonasio focuses heavily on genomics, which he thinks may be the "most transformative new approach in epigenetics." For example, he relied on genomics in recent studies of two ant species, *Camponotus floridanus* and *Harpegnathos saltator*. He reassembled *de novo* high-quality genomes, and then he annotated long noncoding RNAs (lncRNAs). In *H. saltator*, he discovered that the expression of lncRNAs differs across developmental stages, as well as in the brains of ants of different castes.

Epigenetic-Based Drug Development

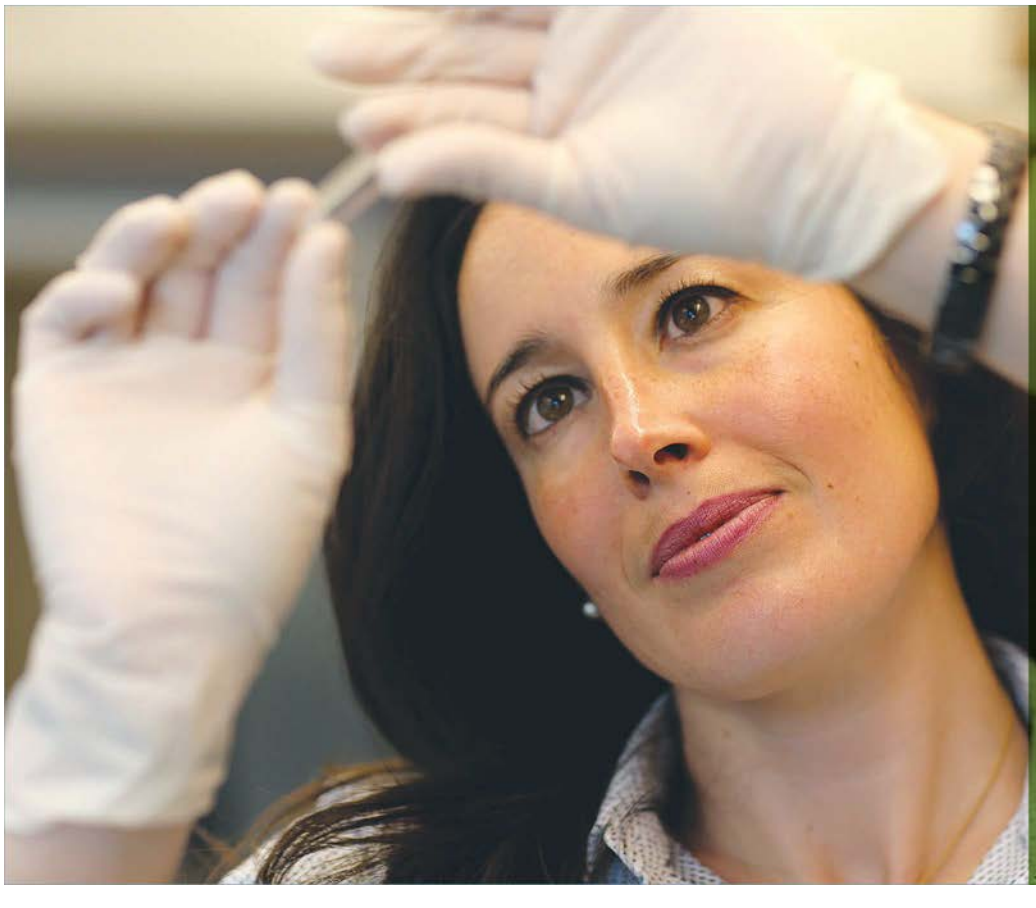
The lab of Giacomo Cavalli, Ph.D., the head of the Institute of Human Genetics at the French National Center for Scientific Research in Montpellier, France, works to understand 3D genome organization and its functional implications. Specifically, the lab studies the molecular mechanisms of two main groups of genome regulatory proteins: the polycomb group, which includes gene-repressing histone-modifying enzymes, and the trithorax group, which includes gene-activating histone-modifying enzymes. These proteins, which have been known for roughly a century, influence the genome's 3D organization.

Functionally, polycomb and trithorax proteins regulate the genes that convey inheritance of chromatin states and orchestrate development. They can also, as Dr. Cavalli discovered in *Drosophila melanogaster*, play a role in transgenerational inheritance of chromatin states.

Besides maintaining basic epigenetic processes, polycomb and trithorax "have major roles in human disease," says Dr. Katz. He adds that "even before the discovery that epigenetic modifications have a role in human disease, early fruit fly investigators had evidence that epigenetic enzymes could be targeted to reverse defects caused by epigenetic perturbations. This is why epigenetic drug targets are so promising."

Dr. Cavalli is quick to point out the advantages to drug design based on epigenetics. He tells *GEN*, "Many cancers and other diseases depend not only on mutations, but mainly on altered levels of expression of epigenetic modulators." Therefore, "an epigenetic drug might correct the problem." He adds that "epigenetic component variations are well tolerated in normal tissues as long as critical thresholds are not crossed, suggesting a relatively low toxicity for these drugs," and that "combination with established drugs acting on other principles is very promising since they leverage different and frequently orthogonal cell processes."

See Epigenetics on page 16



Cigall Kadoch, Ph.D., assistant professor of pediatric oncology at the Dana-Farber Cancer Institute, studies the BAF complex, which regulates chromatin structure. Although the BAF complex has been credited with protecting cells against cancer, aberrant versions of BAF have been implicated in rare, hard-to-treat cancers such as synovial sarcoma and malignant rhabdoid tumors. In a recent study from Dr. Kadoch's lab, BAF complexes were shown to be mutated in more than 20% of human cancers.

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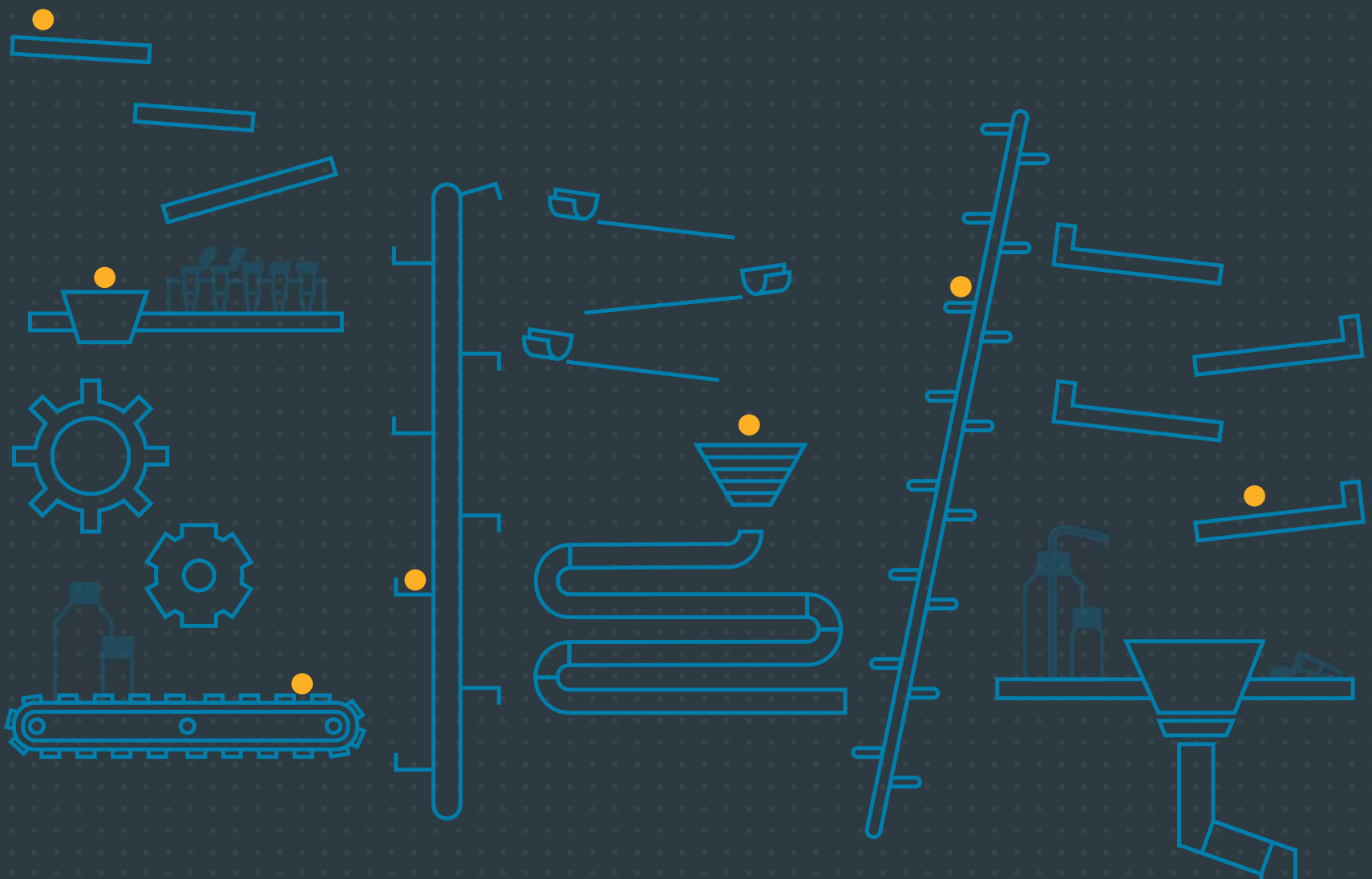

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Tissue Cross-Reactivity Studies Meet with Approval

BioIVT Shows How Preclinical Studies Can Support IND Applications and CTAs

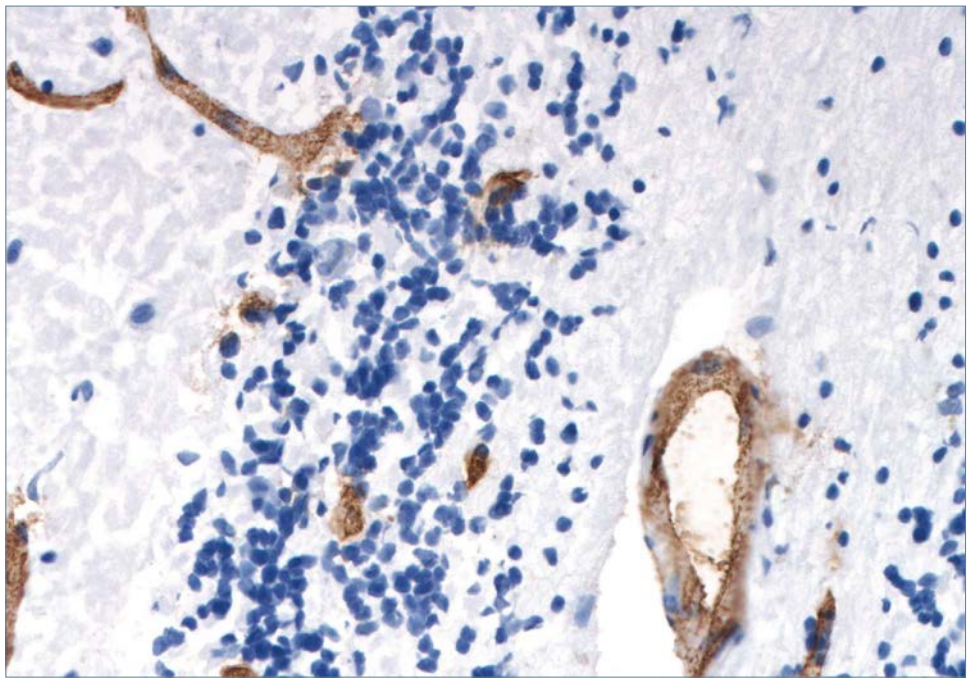

Figure 1A. Confirmatory assays are performed to validate the antigenicity of tissues used in TCR studies. One such test involves the immunostaining of tissues with von Willebrand Factor (vWF) antibodies. Image shows the binding of vWF antibodies to microvascular endothelium proteins in a frozen section of the cerebellum.

Julia Stevens, Ph.D., and Amanda Woodrooffe, Ph.D.

It is extremely costly to have a therapeutic antibody or antibody-like molecule fail during clinical development. Therefore, drug developers need to mitigate any potential issues before first-in-human studies begin. Many preclinical investigations that are essential components of a regulatory Investigational New Drug (IND) application or Clinical Trial Application (CTA) are intended to minimize the risk of harm when a drug is first given to humans. One such preclinical safety study is the assessment of tissue cross-reactivity (TCR).

A TCR study incorporates a series of *ex vivo* immunohistochemical (IHC) screening assays conducted primarily to identify off-target binding, but also to pick up previously unknown sites of on-target binding for a novel biotherapeutic. Essentially, the presence of therapeutic antibody binding in frozen *ex vivo* tissues is used to give an indication of potential organ toxicity *in vivo*.

Whether such staining actually correlates with organ toxicity is still regularly debated, but TCR studies remain a requirement in the data package for an IND/CTA submission for most biotherapeutics. TCR studies are also used to compare staining patterns between human and animal tissue, providing additional justification for the choice of models used to generate other preclinical safety data.

Protocol Development

One of the most important aspects of a TCR study is the development of the IHC protocol. Given that the biotherapeutic to be tested has not been designed as an IHC tool, this poses a technical challenge that can be overcome only by rigorous assay workup. Since a favorable dataset for a TCR study is a negative result, it is very important to know that the assay is specific and robust before the test tissues are examined.

Many researchers underestimate the length of time needed to develop a sound IHC method. Time-pressed researchers may be tempted to cut corners, but they should know better than to risk gathering misleading TCR results when the good laboratory practice (GLP) assays are run.

Test Items

Test Items come in many forms, some differing substantially from immunoglobulins in structure. Irrespective of format, consideration must be given to how the molecule is to be detected in an IHC assay.

Unlabeled human or humanized antibodies can be detected by precomplexing with an anti–human antibody before applying to the test tissues. However, it is technically easier to work with an antibody that has been labeled with a small molecule, such as biotin or fluorescein isothiocyanate (FITC), that can be detected with an antibody specific for the label. Indeed, for many molecules, labeling is a necessity to facilitate detection.

While biotinylation is a well-established and relatively straightforward technique for labeling biological molecules, FITC may be preferable in a TCR study as it avoids the need for additional avidin-biotin blocking steps in the assay protocol, without which background staining of tissue is likely to prove problematic. In either case, it is important to establish the impact of any labeling on the binding properties of the molecule and to ensure test and control items are labeled to the same degree.

Control Items

The inclusion of a Control Item is strongly recommended.[1] The Control Item, essentially an isotype control, is usually a molecule identical in structure to the test item (includ-

Epigenetics

Continued from page 14

However, targeting epigenetic mechanisms for drug development brings its own unique challenges. "For the moment," Dr. Cavalli advises, "specificity is an issue in many cases." Dr. Katz adds that "epigenetic modifying enzymes work on many genes," so "there is the potential to cause changes in other genes that were not originally affected by the disease, potentially leading to unanticipated side effects." Dr. Cavalli cautions that "these drugs will have to be carefully tested and not blindly transported from the treatment of one to another disease."

As for future developments, Dr. Cavalli says to look for "more selective and sensitive histone methyltransferase inhibitors, particularly for EZH2." He adds that we will likely see "improvement in BET inhibitors and in DNA methylation, with TET inhibitors as well as mutated IDH inhibitors to watch out for." He adds that "modulators of SWI/SNF complexes are also quite exciting."

This last point, he suggests, would meet with the approval of Cigall Kadoch, Ph.D., assistant professor of pediatric oncology at the Dana-Farber Cancer Institute. As it happens, when *GEN* caught up with Dr. Kadoch, she concurred with Dr. Cavalli.

A New Class of Epigenetic Targets

When Dr. Kadoch joined Stanford University's Crabtree Lab, it was not focused on the study of cancer. Rather, it was devoted to improving our understanding of development and differentiation. Specifically, it was scrutinizing the chromatin remodeling complexes called BAF complexes and their role in gene regulation.

The genomic sequencing projects from the last decade have brought BAF complexes to the forefront of attention, owing to the fact that they are mutated in over 20% of human cancers. Dr. Kadoch tells *GEN* that "it was a very surprising and exciting discovery that perturbed BAF complexes were major drivers of cancer, given that for years, BAF complexes had been thought to mainly serve maintenance functions in the cell."

Today, perturbed BAF complexes seem to be one of the biggest culprits in cancer. Indeed, "they are the most frequently mutated chromatin regulator in all of human cancer," asserts Dr. Kadoch. In some cancers, such as synovial sarcoma (SS), a rare malignancy, 100% of tumors have a precise translocation involving a specific BAF subunit, SS18, indicating that the translocation is the initiating oncogenic event.

Interestingly, no other mutations are found in these tumors. Mutations in the BAF complex have been found in many pediatric and adult cancers, ranging from rare sarcomas to common cancers such as lung and breast cancers. Unlike HDACs and polycomb complexes, to date, there are no agents in the clinic targeting BAF complexes. Dr. Kadoch says that it is "an unmet need."

With a desire to see her laboratory's discoveries translated into new medicines, Dr. Kadoch founded **Foghorn Therapeutics** to "hunt for drugs." The company, which has raised $50 million from Flagship Ventures, plans to introduce drugs to the clinic by 2020. It will work on both cancer targets and the other diseases in which BAF complexes have been implicated, including autism spectrum disorder and intellectual disability syndromes.

The power of epigenetics in drug discovery is only beginning to be uncovered. The last two decades of basic research and the tools that have come out of the genomic revolution have positioned epigenetics at the forefront of novel treatments for cancer and other diseases. As Dr. Cavalli tweeted, "Is there anything really beyond epigenetics? Epigenetics is like the Pillars of Hercules: Sailors who try to go beyond sink into the realm of Atlantis and are lost forever..." GEN

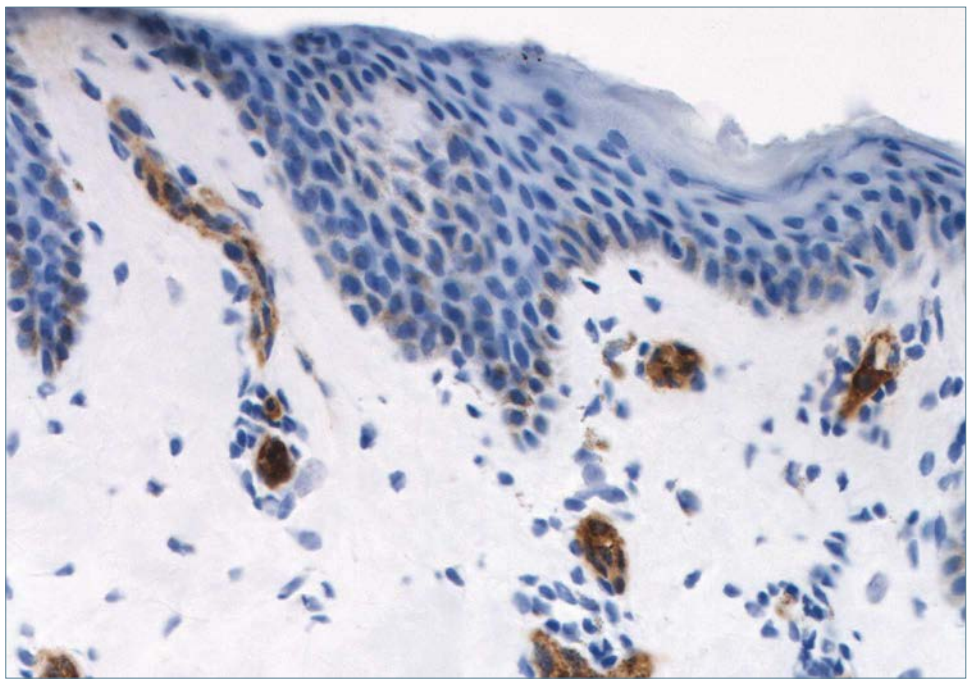

Figure 1B. Image shows the binding of vWF antibodies to microvascular endothelium proteins in a frozen section of skin.

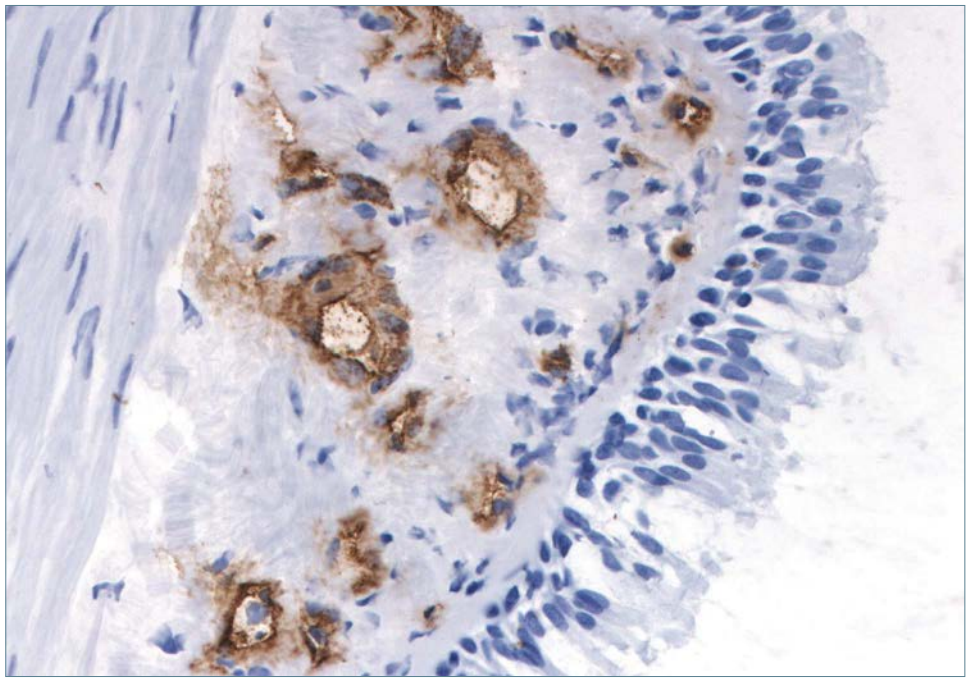

Figure 1C. Image shows the binding of vWF antibodies to microvascular endothelium proteins in a frozen section of the bronchus.

ing labels such as biotin or FITC) but raised against a molecule unlikely to be found in human tissue—for example, green fluorescent protein, a plant protein, or even snake venom. Ideally, such a molecule should be prepared in parallel with the Test Item and used to ascertain the background level and pattern of tissue binding that occurs irrespective of the complementarity-determining region (CDR).

Failure to appreciate the value of including a suitable Control Item for comparison may result in any binding of the Test Item being mistakenly interpreted as specific and therefore indicative of potential organ toxicity. There are other ways that binding specificity can be assessed, for example, competing out binding by preincubation with a molar excess of soluble antigen. However, these methods add to the overall study cost, and sufficient soluble antigen is not always available.

Positive Control Material

Selection of suitable positive control material is crucially important for TCR protocol development, as well as for use in GLP studies, to validate the test item in all of the assay runs—which are likely to be numerous.[2] The positive control material needs to be representative of the frozen tissue that the test item will encounter in the full GLP assays.

Using overexpressing cell lines or other types of positive control material is inferior to using frozen tissue, which retains tissue matrix. Where no suitable tissue exists that naturally expresses the target of interest, various alternative techniques have been attempted, including subcutaneous injection of antigen-coated beads prior to harvesting skin from mice.

To address this issue, we developed a proprietary method by drawing on our expertise in handling human tissue. Our method allows incorporation of soluble antigen into a human tissue matrix, resulting in a frozen, sectionable material suitable for use as a positive control material when no other solution exists.

Test Tissues

The quality of the frozen tissues used in GLP TCR studies is of the utmost importance. Whether from postmortem or surgical origin, human tissues must have good morphological preservation to allow adequate interpretation of staining patterns and crucially must retain antigenicity. Confirmation of tissue antigenicity as part of the GLP study is strongly recommended, as testing prior to the GLP study does not guarantee antigenicity as a sample is sectioned through (*Figures 1A–1C*).

Interpretation of Results

A qualified pathologist should interpret the results. Staining observed with the Test Item should be compared to that seen in adjacent sections incubated with the Control Item, and specific staining should be considered only where Control Item staining is not present. The pathologist may, in addition, make a judgment as to whether smeared or very diffuse staining is specific in nature.

The cellular location of specific staining is also important to consider, since staining of cytoplasm, which is unlikely to be accessible to a biotherapeutic *in vivo*, is less likely to translate into a biological effect than membrane staining, so may be less of a concern from a safety perspective.

The biological relevance of any TCR staining can never be fully determined until other human safety/toxicity data (for example, from clinical trials or post-marketing surveillance) become available, so it is important to interpret data with caution. TCR studies are here to stay, at least for now. It will be interesting to see what regulatory bodies, such as the FDA and EMA, will require for tissue cross-reactivity assessment as newer technologies become available. GEN

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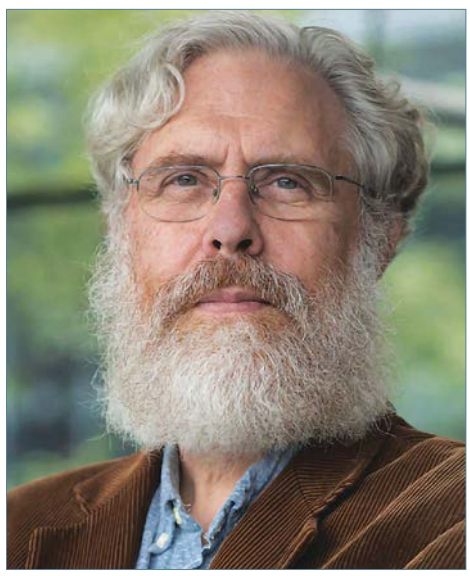
University Flunk-Out to Genomics Pioneer*

An Interview with George Church, Ph.D., by James M. Wilson, M.D., Ph.D.

Dr. Wilson: How do you think genome editing, in which you played an important role, will be most impactful in the near future?

Dr. Church: I saw genome editing up close and personal since the early '80s when we were interested in homologous recombination. I joined Gail Martin's lab to apply homologous recombination to mouse embryonic stem cells. But Mario Capecchi and Oliver Smithies got there first. What we do today with CRISPR is in some ways less impressive because those groups focused on precise editing, whereas much of the current CRISPR craze is sloppy genome vandalism.

In 1999–2001, my lab worked on custom zinc fingers. I also advised a startup called Gendaq, cofounded by Yen Choo and Aaron Klug, which merged with **Sangamo**. Bernard Dujon, who was one of my mentors as a graduate student, developed meganuclease technology with Andre´ Choulika.



George Church, Ph.D.

Dr. Wilson: You have also done work on editing porcine models to address the issue of limited organ transplantation. What is the status of this work and how close are we to addressing this issue?

Dr. Church: The idea of transplanting from animals to humans becomes more attractive as we see more acceptances of allogeneic transplants from humans to humans without regard to initial matching. Now, for the first time we can alter the donor genome, whether it is a human or an animal, to prevent tissue rejection, especially in a properly immune-suppressed patient.

Twenty years ago, the pioneers of xenotransplantation had hoped that one or two mutations would be sufficient to make pig organs compatible with humans, but now dozens of changes are feasible (and clearly needed). In 2015, we simultaneously edited 62 locations in the pig genome over about 14 days. That was surprising to us and remains kind of a record in mammalian editing.

So, I think we are getting close. MGH (Massachusetts General Hospital) and **eGenesis** are collaborating on preclinical trials in non-human primates with some of the early strains.

We don't have the perfect strain yet, but we have almost all of the components in various pig strains and plan to combine them soon. Human trials might be a couple of years away, but they're coming.

Dr. Wilson: You mentioned the role of NGS in prenatal diagnosis and genetic counseling. Where do you see the next round of technological advances with the greatest potential for impact?

Dr. Church: Well, I think it's still important to emphasize the role of prenatal and, ideally, premarital genetic counseling in eliminating genetic diseases. The Jewish organization, Dor Yeshorim, and other genetic counselors have more or less eliminated diseases like Tay-Sachs, preventatively. So, we need to keep this in mind as we develop these initially expensive gene therapies.

I think that other technology advances could focus on developing countries where equitable distribution of orphan drugs is a challenge, even with government subsidies.

One such opportunity is in gene drives, where the therapeutic is spread through an animal vector population, like mosquitos for malaria and white-footed mice for Lyme disease. Such a strategy could benefit both developing and developed countries.

For industrialized nations, the next frontier in gene therapy is longevity and age reversal. We already have vast amounts of information on what causes longevity and aging reversal in model organisms, and hence can now turn this knowledge into gene therapies that simultaneously tackle multiple molecular systems to reverse the aging process.

Dr. Wilson: I want to explore the limits of what is possible with synthetic biology as far as resurrecting extinct species. I know you have been involved in similar ideas so could you tell us what is possible and how far we are from a real "Jurassic Park"?

Dr. Church: I think a good way to frame this topic is around the possible impacts of synthetic biology on agriculture and ecosystems. The excitement surrounding ancient DNA is not so much about bringing back dinosaurs, but more about bringing back diversity from extinct species, because many of the organisms we need for our own human needs are experiencing bottlenecks or are ill adapted.

One of the ecosystems that I am most keen on is the tundra in Canada, Russia, and Alaska, where there are 1,400 gigatons of carbon at risk (possibly the result of bad decisions humans made tens of thousands of years ago). I think we can use new molecular tools to improve those natural environments. We also see an opportunity in using these tools to lessen the impact of unnatural environments, like the gigantic amounts of agricultural land. We have resurrected a number of extinct genes and we hope to apply them as part of the solution toward improving diversity and saving endangered species, like the Asian elephant. GEN

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Innovations in Viral Clearance

Gareth John Macdonald

Viral clearance is not a new challenge for the biopharmaceutical industry. However, regulatory demand for better data combined with the growing recognition of the shortcomings of current virus removal and inactivation methods are fostering innovation, according to industry experts.

Ensuring biopharmaceutical products are free of viruses is vital. The risk that a patient treated with a contaminated product will become infected by the virus is significant, particularly if they are already immunosuppressed.

Viruses can be inadvertently introduced into biopharmaceutical production lines in a variety of ways. For example, they can be present in raw materials or found on improperly cleaned bioreactors and other processing equipment. As a result, manufacturers follow procedures designed to minimize the risk of viral contamination.

However, viruses are resilient and can be difficult to remove, particularly non-enveloped strains.[1] As a consequence, biopharmaceutical producers also implement viral inactivation and or removal/clearance steps to ensure the safety of their products.

GEN interviewed several viral clearance experts who recently spoke at the Cambridge Healthtech Bioprocessing Summit in Boston.

Facility for Viral Clearance

Facility and production-line design can play a vital part in viral clearance strategies, according to Paul W. Barone, Ph.D., associate director of the biomanufacturing research program at the MIT Center for Biomedical Innovation.

He tells *GEN*, "Traditional biotech products, like proteins and vaccines, have well-established processes and approaches to ensure viral safety that have been proven to be effective."

Dr. Barone cites approaches such as solvent-detergent treatment, low pH activation, and nanofiltration as proven, effective ways of removing viruses. "Many traditional manufacturing processes have been validated, in total, to reduce potential viruses by 18 logs or more," he adds.

A theme common to most viral clearance methodologies is to view the process as a continuum, with actions implemented during each unit operation being used to reduce viral load. Such a strategy requires that the manufacturer establishes pre- and post-clearance segregation between each process, which is a technical challenge, according to Dr. Barone.

"Facility segregation is really product and facility dependent and is one part of an overall safety strategy. The most conservative approach is to have the process segregated by walls with separate HVAC systems. This has often been the 'easiest' way to get approval from regulators.

"There are a variety of manufacturing facilities which may have constraints on how segregation can be applied, and certain products will have specific processing requirements as well," he says.

To help address these challenges, Dr. Barone and his colleagues at MIT's Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB) have developed a definition of pre- and post-viral clearance segregation. The definition "would be valuable for the industry and would help companies as they consider their own approach to facility segregation both now and in the future," says Dr. Barone.

The work has been accepted by the *PDA Journal of Pharmaceutical Science and Technology*, but it has yet to be published.

Protein Sciences, a Sanofi company, specializes in vaccine development and protein production. At its laboratory in Meriden, CT, Protein Sciences exploits an expression platform that incorporates insect cells and baculoviruses. This platform offers viral clearance capabilities. It has been qualified for freedom of mycoplasma, spiroplasma, and other adventitious organism contamination, as well as freedom of retrovirus-like particles.

Rational Testing

A common approach to viral clearance is to undertake extremely detailed cell-line and reagent characterization. Such a strategy was core to the development and production of the influenza vaccine, Flublok Quadrivalent, according to Penny Post, Ph.D., vice president at **Sanofi Protein Sciences**.

"The FDA requires extensive safety testing and characterization of cell substrates for the production of vaccines," she tells *GEN*. "We followed the FDA's guidance document, 'Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications' to qualify our cell line," she adds.

To achieve this, Dr. Post and her team performed general adventitious agent testing using traditional *in vitro* and *in vivo* methodology set out by the FDA as well as PCR-, biochemical-, and infectivity-based assays for any viruses of concern identified from literature searches.

The group also conducted viral clearance studies to determine how effective purification processes used in the production of Flublok were at removing a selection of model viruses.

"Model viruses were selected to represent a wide range of potential contaminants, including DNA-based, RNA-based, enveloped, non-enveloped, small, and spherical, icosahedral, and rod-shaped viruses," Dr. Post says.

Expression System Selection

The cell line used can also be used to minimize the viral clearance work required. For example, the protein components from which Flublok is made are produced using a baculovirus-based expression system which, according to

See Viral Clearance on page 22

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Viral Clearance

Continued from page 20

Dr. Post, was selected in part to make viral clearance more straightforward.

The process involves culturing the production cell line in a bioreactor, infecting the cells with a baculovirus engineered to contain the genes of interest to be expressed, incubating the infection for the appropriate amount of time to obtain high product yields with minimal host cell proteins, and purifying and formulating the expressed product.

"The baculovirus infection is a lytic process and is highly species specific; the virus cannot replicate in mammals and therefore has even been used as a biopesticide," says Dr. Post.

Model Viruses

Mock viruses—such as those used by Dr. Post and her team—are a recent tool to emerge to help pharmaceutical companies fine-tune viral clearance processes. The basic idea is to deliberately introduce virus-like particles (VLPs) into a manufacturing set-up to determine the efficacy of established removal procedures to allow whatever fine-tuning is necessary.

MockV Solutions is seeking to tap into this growing area of demand. CEO David Cetlin tells *GEN*, the idea to produce VLPs for clearance testing was prompted by experience of the shortcomings of current testing methods.

"While working as a downstream process development scientist, I was frustrated that often we'd spend years developing and optimizing a scale-down manufacturing step only to see it underperform during viral clearance validation.

"I thought there must be something commercially available that could serve as a predictor for live MVM and other model viruses. At some point it hit me that VLPs could be used for this purpose and I started MockV Solutions," Cetlin says.

He claims that, in contrast to costly live virus spiking studies that require expertise and take months to design and analyze, MockV's VLPs could allow for the same data to be generated in a fraction of the time and at a lower cost.

David A. Cetlin at work in MockV Solutions' lab in Rockville, Maryland.

Development work is ongoing, according to Cetlin, who says, "Our current prototype kit, which predicts MVM clearance, is limited by the sensitivity of the MVP quantification assay. As a result, an end user can expect to achieve less than or equal to 3.0 logs of reduction, based on their experimental parameters."

He adds, "We are currently working to improve the assay, increasing its dynamic range to 4–5 log10. This work is being funded through an SBIR grant with the National Center for Advancing Translational Sciences (NCATS) of the NIH."

Cetlin adds that the aim is to make the test kits available to the pharmaceutical industry through a range of channels, including deals with companies in the contract research sector.

"Offering a product or service such as the MVM-MVP Kit would allow a CRO to capitalize and engage customers during these phases leading up to validation studies," he says. "We actually have a service structure worked out with **Texcell** to do just that."

Cetlin explains that customers purchase spiking MVP and ship their samples to Texcell for analysis, which he says allows the CRO to build relationships with these customers who may use them for viral clearance validation. MockV will also seek agreements with technology suppliers, according to Cetlin.

"For industry vendors, such as those selling chromatography resins, virus filters, or analytical kits, a product line of predictive viral clearance kits would be of great interest to their existing customers." GEN

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Hydrogen Bond Chromatography

Attributes Include Salt Tolerance and Ability to Sort Retained Biomolecules by Size

Pete Gagnon

Nobel Laureate Linus Pauling, Ph.D., predicted in 1939 that hydrogen bonding would prove to be more significant in the field of biology than any other type of chemical bond. His prediction has been fulfilled abundantly for the most part, except in chromatography. Hydrogen bonding has been acknowledged as a contributing factor to protein retention on ion exchangers since the mid-1950s and more recently suggested as a selectivity modifier for many mixed-mode chromatography products.[1,2] But it has not yet been exploited as a primary adsorption mechanism.

There are various reasons why it may have been overlooked. The mechanism is less intuitive than methods like ion exchange, and individual hydrogen bonds are known to be weaker than ionic bonds. However, it is also true that potential hydrogen bonding partners on biomolecule surfaces are far more abundant than charged residues. Recent data show that hydrogen bond chromatography is not only feasible but offers unique practical attributes that ion exchange, hydrophobic interaction, and other modes of chromatography cannot. Among them: a high degree of salt tolerance and an ability to sort retained biomolecules by size.

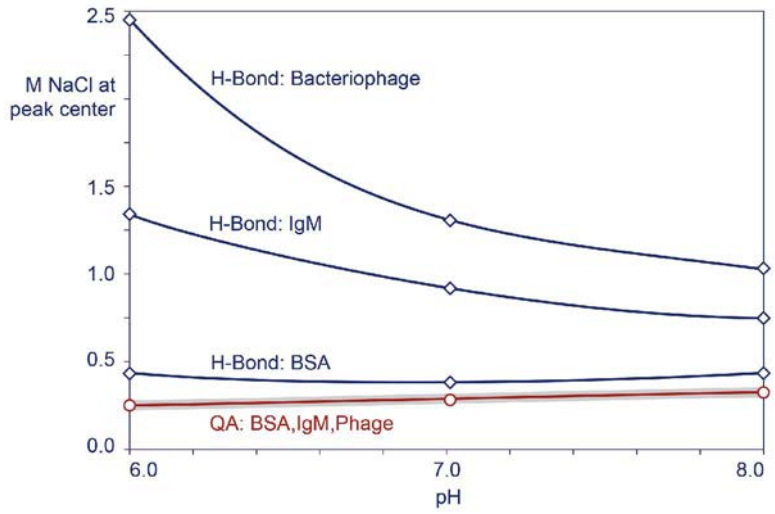

Figure 1. Elution of proteins and virus particles from CIMultus™ H-Bond™ ADC and CIMultus QA as a function of pH.

Hydrogen Bonding Monolith Stronger at Acidic pH

Figure 1 compares retention of a series of purified test samples on a hydrogen bonding monolith (CIMmultus™ H-Bond™ ADC) versus a strong anion exchanger (CIMmultus™ QA).[3] The ligand for H-Bond ADC consists of a terminal series of hydrogen donors grafted to a root series of hydrogen acceptors. It has a weak positive charge, but no cation exchange or hydrophobic groups. The QA (quaternary amino) anion exchanger provides an ideal experimental control because it is also positively charged but it cannot participate in hydrogen bonding. The hydrogen atoms covalently bonded to its methyl carbon atoms are unable to act as hydrogen donors, and its quaternary nitrogen atoms lack the lone pair of free electrons required to accept hydrogen.

Consistent with expectations, retention is weaker for all biomolecules on the strong anion exchanger under acidic conditions (*Figure 1*). Biomolecules become more electropositive at low pH, creating electrostatic repulsion from the positively charged exchanger surface. Directly contrary to those results, retention on the H-Bond column becomes stronger at acidic pH—despite its positive charge. This is understood to result from increasing protonation, which creates a net increase in hydrogen bonding potential.

Another important distinction is that biomolecule retention on the H-Bond column increases with molecular size, and the effect is compounded at acidic pH. At pH 6.0, H-Bond retention of bovine serum albumin (BSA, ~66 kDa) is about twice as strong as the strong anion exchanger. Retention of IgM (~960 kDa) is about four times stronger and the trend continues with the virus at about 16.7 MDa. There was no size discrimination among these species at any pH on the strong anion exchanger. Using the QA monolith as a reference to indicate the contribution of ionic interactions, the implication is that hydrogen bonding is responsible for about 80% of the binding energy achieved at pH 6.0 on the H-bond monolith.

Van der Waals forces could hypothetically contribute to this differential but experiments conducted in the presence of sorbitol suggest that most of the binding energy is attributable to hydrogen bonding. Sorbitol is a monosaccharide with six hydrogen donors and acceptors. As such, sorbitol is to hydrogen bond chromatography as what salts are to ion exchange chromatography. A sorbitol solution of 200 mM reduced binding strength for all tested samples by about half. Experiments with higher concentrations were limited by viscosity of the sugar.

These findings lead to the question of practical utility and there are several features of note. The high salt requirements for elution translate into high tolerance for salts in applied samples. An IgM requiring a NaCl concentration less than 20 mM to bind a strong anion exchanger at pH 6.0 bound effectively to the H-Bond monolith in 200 mM NaCl at pH 6.0. High salt tolerance can also be exploited to minimize or eliminate the need for a separate buffer exchange step in multi-step chromatography methods.

Size Discrimination

The ability of hydrogen bond chromatography to support a degree of size discrimination also creates compelling opportunities to improve purification. *Figure 2* illustrates a series of chromatograms conducted with an IgM mAb in cell culture harvest. All runs were eluted with linear NaCl gradients. Even at pH 8.0, the H-Bond monolith gave clearly better separation from contaminants than the strong anion exchanger. Separation improved further at pH 6.0 with the majority of small contaminants eluting before IgM and larger contaminants, including aggregates and DNA, eluting after.

Stronger binding of larger solutes particularly highlights the suitability of hydrogen bond chromatography for very large biologics, like virus particles, but these products also impose special requirements. Diffusion constants for viruses can be 5–15 times slower

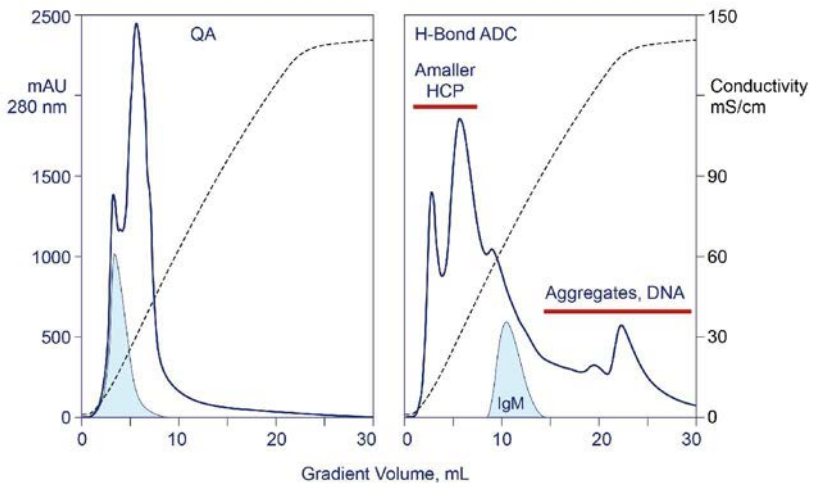

Figure 2. Comparative fractionation of an IgM mAb from cell culture harvest on CIMultus QA and CIMultus H-Bond ADC.

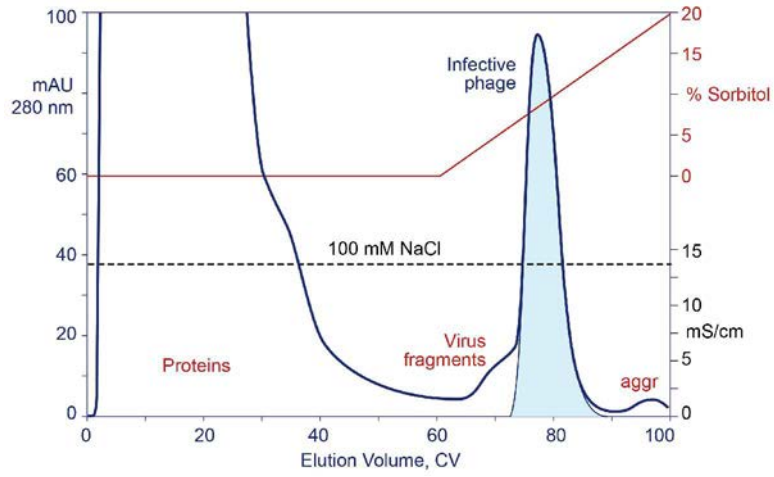

Figure 3. Fractionation of bacteriophage from cell culture harvest by CIMultus H-Bond ADC with a sorbitol gradient at pH 6.0.

than for proteins like albumin and, in many cases, they are too big to enter the pores in traditional column-chromatography media. These factors limit binding capacity and productivity. Their large size also makes them highly vulnerable to shear forces such as created in the void volumes of particle columns.

Monoliths bypass these concerns. Mass transport is exclusively convective through highly interconnected 2–6 µm channels. Flow is laminar, thereby avoiding the turbulent shear stress created in particle columns. Capacity and separation performance are both independent of flow rate, so neither capacity nor productivity are compromised. Virus capacity on monoliths is commonly 10–100 times higher than on particle columns.[4]

Development of Hydrogen Bond Chromatography Methods

Salt gradients represent the simplest screening option but alternative gradient formats offer better overall contaminant reduction. Gradients with nonionic hydrogen donor-acceptors, like sorbitol, represent one option and they offer the additional benefit of stabilizing most biomolecules. *Figure 3* illustrates a bacteriophage eluted in a gradient to 200 mM sorbitol at 100 mM NaCl, pH 6.0. In the absence of sorbitol, its elution required nearly 2.5 M NaCl.

Another option is to elute with an increasing pH gradient. A monoclonal IgM that required 1.2 M NaCl for elution at pH 6.0, eluted in a gradient from pH 6.0 to pH 8.0 at 50 mM NaCl. Subsequent analysis by size exclusion chromatography (SEC) showed a single IgM peak free of aggregates. These results are further noteworthy because they highlight the dominance of hydrogen bonding as the primary adsorption mechanism, and its distinction from anion-exchange chromatography. pH gradient elution from anion exchangers is achieved with *decreasing* pH gradients.

These results frame a general strategy for method development. Screen first with a salt gradient. If it is a particular objective to maximize size discrimination, begin at the lowest pH where the product of interest is known to be stable. Otherwise, pH 6.0 is a good starting point. Evaluate elution with a pH gradient or nonionic donor-acceptor gradient at the highest salt concentration where the product of interest does not elute. Hold salt concentration constant during pH or nonionic donor-acceptor gradients. As with other chromatography methods, design of experiments can be used to reduce the workload of optimizing the fine details.

Overall, expect hydrogen bond chromatography to be complementary to other chromatography methods, and in some cases to offer better performance than established methods. Situations where size discrimination is helpful will be prime applications. Being able to conduct separations in a chemically biocompatible low-shear environments will be especially attractive with very large biologics. Otherwise, the unique selectivity of hydrogen bond chromatography may provide the needed missing ingredient in any analytical or preparative situation. GEN

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Horseshoe Crabs Are at Risk– so Endotoxin Tests Are, Too

Protect the Living Fossil, Lonza Urges, and Qualify Animal-Free Testing Alternatives

Robert Porzio

The presence of harmful bacteria in parenteral drugs or implantable devices can cause inflammatory responses such as fever and, in some cases, may even be fatal. As a result, robust bacterial endotoxin testing (BET) workflows are employed by the pharmaceutical industry to help ensure the production of safe and effective products.

These tests enable the detection of endotoxins, the pyrogenic components found in the outer membrane of Gram-negative bacteria. In 1977, the FDA approved the use of the limulus amebocyte lysate (LAL) test in the United States and tachypleus amebocyte lysate (TAL) test in Asia, to replace the rabbit pyrogen test for endotoxin detection in pharmaceuticals and medical devices.

These gold-standard tests rely on cells derived from the blood of horseshoe crabs, so-called living fossils whose ancestors date back over 450 million years—200 million years before dinosaurs existed. The horseshoe crab's amebocyte cells bind to endotoxins, initiating a blood-clotting cascade that includes three inactive enzymes and a clottable protein called coagulogen. The coagulogen proteins stick together to form a clot or gel that may be detected by fluorescence.

With a growing focus on the development of innovative biotherapeutics that have a higher risk of endotoxin contamination and the advent of personalized medicine requiring individual drug testing, endotoxin testing workflows are becoming more important than ever. However, relying purely on the horseshoe crab to provide this crucial safety test simply isn't sustainable. In this article, we consider why protection of the horseshoe crab is so important for our own health, and how the validation of alternative endotoxin testing approaches offers a more sustainable solution.

Seeing the Threat to Horseshoe Crabs

Spanning four distinct species (the North American *Limulus polyphemus* species and the Asian *Tachypleus tridentatus*, *T. gigas*, and *Carcinoscorpius rotundicauda* species), horseshoe crabs have a fascinating, almost alien-like anatomy, including blue blood, five pairs of legs, a mouth between their legs, and 10 eyes located around their body. They also have a long tail known as a telson.

Horseshoe crabs use the telson for steering and to help flip themselves upright when they have been turned upside down. This anatomical feature is important for the survival of horseshoe crabs, which commonly get overturned by high wave action during spawning. Failure to right themselves often results in the death of the animals, either from prolonged exposure to heat and air or from predators such as seabirds who target them for food.

However, in addition to natural perils, horseshoe crab populations are increasingly threatened by human activity. Their natural habitat is being degraded by the development of man-made infrastructures such as bulkheads, groins, revetments, and seawalls on U.S. beaches. Horseshoe crabs are also vulnerable to overfishing.

Since the 1970's, the American horseshoe crab has been harvested in substantial numbers (approximately 500,000 annually) for bait in the American eel and conch fisheries. Two of the three Asian species of horseshoe crabs (*T. tridentatus* and *T. gigas*) are consumed throughout coastal regions of many Southeast Asian countries. While precise numbers are unknown, estimates suggest it is in the hundreds of thousands annually.

Although the biomedical use of the crabs for endotoxin testing has increased, it has remained stable in the United States over the last few years, with an estimated 430,000 crabs in the United States brought to biomedical facilities in 2016.[1] The horseshoe crabs are usually unharmed during the blood collection. According to the Atlantic States Marine Fisheries Commission, all but 15% of bled crabs survive to be released back into the sea.[2]

While a similar number of horseshoe crabs are harvested each year in Asia, the crabs are not generally returned to the sea afterward. Instead, once the blood has been extracted, their body parts are sold for human consumption, and the shell dried and sold for chitin. Consequently, the populations of the three Asian horseshoe crab species are in decline, a situation which will ultimately lead to a greater reliance on the North American horseshoe crab species.

Moreover, if vaccine production continues to grow at current rates (particularly in the Asia-Pacific market), this could place a more significant strain on lysate resources. Additionally, the rise of personalized medicine could necessitate individual product testing on a per use basis. This could put further pressure on the supply of lysate-based assays.

If the species numbers were to decline further, it wouldn't just be an issue for conservationists, but for everyone who is dependent on the use of pharmaceutical products and medical devices. Protection of the global horseshoe crab popula-

The horseshoe crab's amebocytes, or blood cells, contain a clotting agent that forms in the presence of Gram-negative bacteria. This agent, which is used to detect endotoxin in pharmaceutical and medical device products, may become scarce if horseshoe crab populations continue to decline. © Lonza, Basel, Switzerland.

tion is therefore essential for the continued safeguarding of human health.

Safeguarding the Horseshoe Crab

Given the importance of protecting this important species, there are ongoing efforts to develop adaptive-management plans to regulate horseshoe crab harvests. In the United States, the Atlantic States Marine Fisheries Commission regulates the use of horseshoe crabs. However, Asia lacks a coast-wide regulatory body and has minimal in-country harvesting restrictions. Without a centralized governmental body to regulate and enforce harvesting strategies, it is more difficult to institute meaningful change.

The nonprofit Ecological Research and Development Group (ERDG) is dedicated to the conservation of all four horseshoe crab species inhabiting the U.S. and Asian waters. It has initiated the Horseshoe Crab Conservation Network and a host of community-building initiatives including the Backyard Stewardship community sanctuaries and Young Voices: Horseshoe Crabs and the Arts Competition. The ERDG also leads campaigns to encourage the use of alternative bait and gear and reduce human consumption. To date, more than 16 miles of prime horseshoe crab spawning habitat in the United States have been protected through these projects.

Conservation is also recognized and supported by commercial companies worldwide. An annual Global Endotoxin Testing Summit organized by Swiss pharmaceutical manufacturer Lonza brings together scientists, lab managers, and regulators to discuss pertinent topics in endotoxin testing, such as new regulatory guidelines as well as alternative endotoxin testing approaches. The delegates also get a unique opportunity to experience hands-on conservation work in support of ERDG's Just Flip 'em!® program, which encourages individuals to turn over stranded horseshoe crabs during spawning season.

Finding Alternative Endotoxin Tests

However, even with these many conservation programs, it is widely accepted that the bleeding of horseshoe crabs is not a sustainable solution for endotoxin testing. Therefore synthetic, recombinant alternatives to animal-based BET testing workflows are needed.

One animal-free alternative to LAL, known as the recombinant factor C (rFC) assay (**Lonza**), has recently been accepted by the FDA, the European Pharmacopeia, and other leading regulatory authorities as an alternative endotoxin testing method. This assay is based on a synthetic equivalent of factor C, which is the first component of the horseshoe crab blood-clotting cascade that is activated in response to endotoxins.

The test uses rFC to cleave a fluorogenic substrate and subsequently reveal a measurable fluorescent signal. Data from pharmaceutical companies trialing the test has indicated that the endpoint fluorescence technique using rFC is robust, accurate, and precise, and is equivalent or superior to the LAL method of endotoxin testing for various drug products. Other approaches, such as the monocyte activation test (MAT), an *in vitro* pyrogen test that measures the cytokine secretion from human blood cells in response to endotoxins and other pyrogenic substances in the test sample, also do not require the use of horseshoe crab blood.

Despite the availability of these alternative tests and the robust data obtained, efforts to establish replacements for the gold-standard LAL/TAL assays have proceeded cautiously, as new tests must be validated against previous techniques for existing drugs. However, the addition of these extra validation steps to testing workflows can be achieved in as little as three days. Manufacturers of these alternative tests, such as Lonza, have developed protocols for easy adaption and submission. This will also provide additional regulatory support services, making it simple to submit the details needed to meet pharmacopeia requirements.

Ensuring a Sustainable Future

Over the last 10 years, the global pharmaceutical market has grown considerably and is now worth an estimated $1 tillion, compared to approximately $660 billion in 2006. Much of this growth has been driven by the rise in the number of biotherapeutics that are particularly vulnerable to bacterial endotoxin contamination and rely on robust testing workflows.

To protect the horseshoe crab and help ensure a sustainable source of the endotoxin testing agent that is found in the crab's blood cells, Lonza supports conservation efforts and advocates the adoption of best practices for harvesting. The company is also leading efforts to mainstream alternative, animal-free endotoxin tests. © Lonza, Basel, Switzerland.

While strengthening global conservation strategies and boosting awareness will go some way to creating an environment for the horseshoe crab to thrive, validating an alternative endotoxin test that does not rely on this important animal will be key to ensuring a sustainable future for both modern medicine and the horseshoe crab species. Biomedical and pharmaceutical companies appear increasingly willing to devote time and resources to qualify an alternative endotoxin test as regulatory acceptance of other methods grows.

It is hoped that the benefits of the alternative tests and the potential for short validation times will encourage large pharmaceutical manufacturers to invest in replacing the current LAL/TAL tests. However, to achieve this, all the relevant stakeholders, including regulatory bodies, pharmaceutical manufacturers, reagent vendors, academic researchers, and conservationists must work together. GEN

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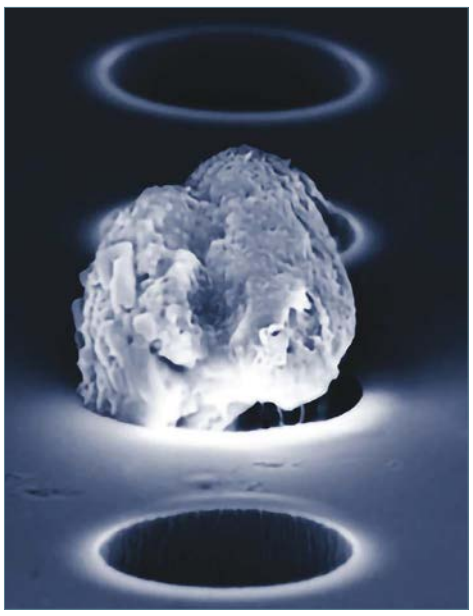
Circulating Tumor Cells beyond Counting

Continued from page 1

bruising experiences such as being targeted by immunoaffinity assays or marked by immunostaining procedures.

Several technologies capable of treating CTCs more tenderly were presented at a recent conference, "Circulating Tumor Cells: Understanding Their Biology and Clinical Significance." This event, which was organized by the Cambridge Healthtech Institute and held in Washington, DC, emphasized that complexities such as CTC heterogeneity, with respect to both molecular markers and biophysical properties, need to be better understood if CTCs are to guide diagnoses and predict patient outcomes.

CTCs, or cells shed by a primary or metastatic tumor into the vasculature or lymphatics, are present at very low levels. Some types of CTCs are believed to be responsible for the spread of cancer, and they may become targets for individualized cancer treatments. However, little is known of the exact composition of CTCs, or the characteristics that distinguish CTCs of different types from each other. Detailed characterization of CTCs could lead to a better understanding of the drivers of metastasis.



At the University of Miami, researchers in the laboratory of Richard J. Cote, M.D., are developing next-generation filter technology for the capture of viable circulating tumor cells (CTCs). By introducing novel pore architectures such as "slot" pores and double-membrane microfilters, the researchers can avoid procedures that complicate functional characterization, such as fixation in 1% formalin.

Label-Free Technology

Methods of confirming whether a cell is really a CTC or not were discussed by Siva A. Vanapalli, Ph.D., professor, chemical engineering, Texas Tech University. Existing methods, Dr. Vanapalli complained, are destructive to cells. These methods make use of immunostaining, which opens the cell membranes, killing them, ruling out their use in downstream applications.

Dr. Vanapalli suggested an alternative approach in his presentation, *Microfluidics and CTCs: Detection, Metastatic Insights and Drug Testing. Microfluidics*, he pointed out, may be used for label-free detection of tumor cells in blood. He described how his group combines inline digital holographic microscopy (DHM) with machine learning technology. The group uses inline DHM to obtain a fingerprint of every cell that flows through a microchannel, and it uses machine learning to distinguish tumor cells from background blood cells.

With inline DHM, a laser is directed onto the cell. The cell scatters the beam, and the interference of the incident laser beam and the scattered beam gives rise to diffraction patterns around the cell.

"We're using light to look at the scattering pattern of the cell, and we use that to decode whether it's a CTC or not," explained Dr. Vanapalli. Early studies of cancer cells spiked into blood samples have shown that inline DHM can detect the cancer cells and differentiate them from other blood cells.

Another potential label-free identification approach is based on the deformability of the cell. When the cells are shed from the primary tumor and enter the circulatory system, they pass through narrow capillary vessels. The cells also deform when they pass through microfluidic devices, which may be used to obtain measures of deformability—measures that may serve as CTC markers.

According to Dr. Vanapalli, the key challenges of CTC analysis may be summarized as follows: First, figure out how to isolate CTCs in a label-free way. Second, figure out how to do drug assays on these cells. "That's how we can really benefit the patient," maintained Dr. Vanapalli. "I'm skeptical of using CTCs as a diagnostic tool, but they can have significant prognostic value."

Microfilter-Based Capture

CTCs and background cells differ with respect to size, a quality that several laboratories are trying to exploit in novel detection, separation, and identification technologies. Size-based technologies could replace conventional alternatives such as immunoaffinity assays, which use antibodies to bind to antigens expressed on cell surfaces. The antibody-antigen interactions allow cells to be removed from the blood and concentrated. Size-based technologies might also be preferable to other biophysical methods, such as those which rely on density gradients or electrical gradients.

At the "Circulating Tumor Cells" conference, the advantages and disadvantages of size-based technologies were highlighted by Richard J. Cote, M.D., professor of pathology, biochemistry, and molecular biology, University of Miami. His presentation, *Capture, Interrogation, Imaging, Automated Analysis and Culture of CTCs: Strategies for the Development of a Transformative Tool to Understand Cancer*, described a microfilter approach for separating CTCs.

He pointed out that cancer cells from solid tumors are larger than almost all normal cells found in the blood. According to Dr. Cote, normal red blood cells range from 5 to 7 microns, whereas tumor cells are 15 microns or more.

"What my lab developed," said Dr. Cote, "was a way to produce microfilters that create a very standard and consistent pore size and distribution that allows for the capture of tumor cells, but almost all normal cells in the blood pass through." Dr. Cote asserted that his method will capture 90 to 95% of tumor cells in a blood sample. The microfilter is made of a plastic material called parylene, which is optically transparent.

"Once we capture a CTC," he explained, "we can analyze it on the filter, just as you analyze a microscopic slide."

Unlike affinity-based technology, which is specific to a particular kind of cancer, Dr. Cote's technology can be used for any type of solid tumor. His group has tested it in breast, colorectal cancer, kidney cancer, melanoma, prostate cancer, lung cancer, and other cancers.

In addition to capturing tumor cells, the filter can capture cancer-associated fibroblasts (CAFs). "Circulating CAF cells are associated with the stage of disease," said Dr. Cote. "In addition to that, in a mouse model of breast cancer, we have shown that the presence of CTCs and circulating CAF cells is associated with progression of disease. The highest association of progression is when you find the co-clusters of CTCs and circulating CAF cells together in association with one another."

Dr. Cote said that his group is investigating ways to automate the technology and developing novel methods to grow CTCs from patients' blood.

Labyrinthine Passages

Another approach to size-based separation of CTCs was described by Sunitha Nagrath Ph.D., associate professor of chemical

Nuclear AR-V7 Protein Expression in CTCs Validates as a Predictive Biomarker

Ryan Dittamore

In the last eight years, five new FDA-approved drugs have demonstrated an improvement in overall survival (OS) for patients with metastatic castration resistant prostate cancer (mCRPC), yet most patients only receive two or three of these therapies.

The question of how to sequence the therapies to achieve the best possible outcome in an individual patient is one of the most important clinical decisions faced by physicians treating mCRPC. In particular, doctors need to decide which patients should receive a less toxic, oral androgen receptor (AR)-directed therapy and which ones should receive a more toxic, cheaper, IV chemotherapy.

To address this question, **Epic Sciences** has developed a nuclear AR-V7 blood-based test that has the ability to predict which patients will not respond to AR-directed therapy and have better OS with chemotherapy. The test examines the expression of the AR-V7 protein in the nucleus of circulating tumor cells (CTCs) in a peripheral blood draw from patients at the time of therapy change. The test is powered by the Epic Sciences No Cell Left Behind™ technology that is precise enough to identify and characterize all the CTCs in a sample, and link their response to a range of drug classes.

A study to clinically validate AR-V7 was performed by Howard Scher, M.D., and his team at Memorial Sloan Kettering, along with other collaborators. Results showed that nuclear localized AR-V7+ patients had longer OS when the patient switched to chemotherapy. Patients who were negative had equal or better survival when treatment with an AR-directed therapy was continued. Importantly, the test has been validated as a predictive biomarker for therapy selection in patients with mCRPC.

The test, available through **Genomic Health** and Epic Sciences as Oncotype Dx AR-V7 Nucleus Detect, has received draft coverage from Centers for Medicare & Medicaid Services (CMS) and is expected to be the first broadly utilized and reimbursed CTC test for clinical decision making in oncology.

Ryan Dittamore is chief of medical innovation at Epic Sciences.

engineering, University of Michigan. Dr. Nagrath said that when her group looked at size-based separations for CTCs, it determined that the methods that are being used, such as centrifugation, lack continuous throughput. Other size-based methods, such as filtration, have disadvantages such as clogged pores, pressure issues, and low throughput.

In her presentation (*Microfluidic Labyrinth Chip for Monitoring Cancer Stem Cells*), Dr. Nagrath described how she developed an alternative size-based method. She developed a microfluidic chip that was inspired by a legendary labyrinth, the one used to imprison the Minotaur. Dr. Nagrath's labyrinth slows down the larger cells while letting the smaller ones move through more quickly.

The labyrinth is 500 microns wide and 100 microns high. It has 11 loops, 56 corners, and a total channel length of 637 nm. The channels, which have lengths ranging from 100 to 700 microns, allow blood to flow through at 2.5 mL/minute. "That's a huge throughput for a microfluidic device," Dr. Nagrath emphasized.

The labyrinth design includes curved channels and sharp corners to create a focusing effect. "Bigger cells experience different forces than smaller cells," Dr. Nagrath explained. "Based on the forces they experience, they get different streamlines in the device, and we collect cells from the different streamlines."

A CTC analysis on blood samples from 20 patients with pancreatic cancer yielded about 50 pancreatic CTCs/mL of blood, with less than 2 CTC-like cells/mL in healthy control samples. In another test in breast cancer patient samples, the average number of CTCs was 9.1/mL.

Dr. Nagrath said that an important advantage of the technology its ability to "focus" cells, achieving CTC isolates of higher purity by excluding contaminating cells. Applications for the isolated CTCs thus far have included looking for mutations in lung cancer for potential targeted therapies and expansion of the cells.

At present, Dr. Nagrath is working on incorporating the labyrinth device into some clinical studies. She said that the University of Michigan has two ongoing clinical trials that incorporate CTCs as a biomarker. Dr. Nagrath added that her group would also like to establish collaborations in breast and prostate cancer.

RNA-Based CTC Signatures

In assessments of cancer, a well-known kind of liquid biopsy focuses on circulating tumor DNA (ctDNA). This kind of liquid biopsy is attractive for several reasons, not the least of which is the ease with which ctDNA can be isolated. Although CTCs are harder to isolate, they can provide a basis for liquid biopsies, too. Despite this difficulty, CTC-based liquid biopsies may offer advantages over ctDNA-based liquid biopsies.

This possibility was discussed by David Miyamoto, M.D., Ph.D., assistant professor of radiation oncology at Harvard Medical School and Massachusetts General Hospital. In his presentation (*RNA-Based Circulating Tumor Cell Signatures for Precision Cancer Medicine*), Dr. Miyamoto emphasized that ctDNA- and CTC-based liquid biopsies could complement each other.

According to Dr. Miyamoto, a limitation of using ctDNA is that it offers a limited view. It looks only at DNA. Using CTCs, however, offers access not only to DNA, but also to RNA and protein. "You get a better understanding of the biology of the tumor as it enters circulation," Dr. Miyamoto insisted.

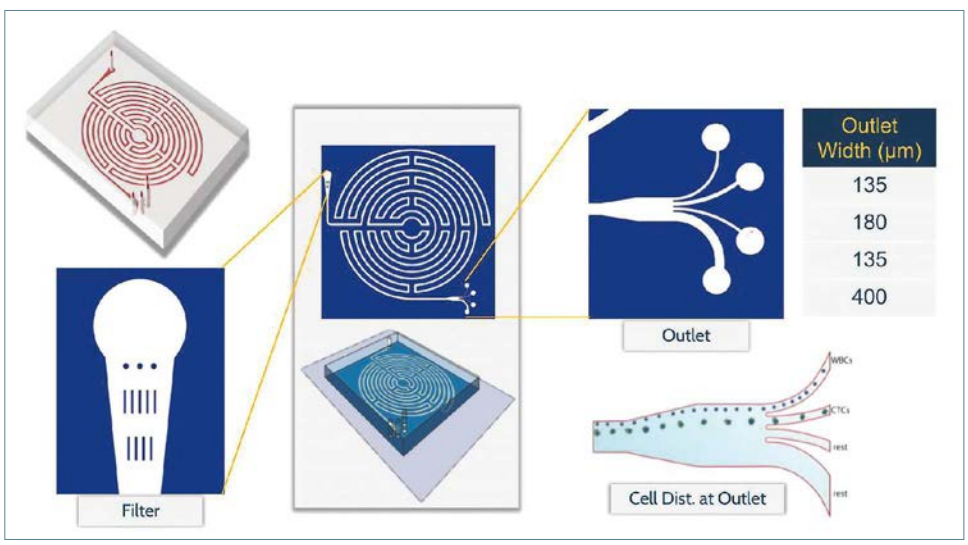
In collaboration with a multidisciplinary team of bioengineers, molecular biologists, and clinician scientists at Massachusetts General hospital, Dr. Miyamoto's group developed a microfluidic technology to isolate CTCs from blood. "Not all CTC isolation technologies are equal," Dr. Miyamoto noted. "One of the main limitations of the technologies out there is that RNA is often not very well preserved in CTCs." RNA is a labile molecule that degrades quickly.

The Massachusetts General Hospital scientists developed the CTC iChip, a microfluidic device that separates CTCs while leaving the cells intact and unlabeled. By sparing the CTCs rough treatment, the CTC iChip preserves CTC RNA.

In a recent study in prostate cancer, the scientists developed an assay using digital PCR to study specific genes expressed in prostate cancer. In patients with metastatic castration-resistant prostate-cancer, they were able to use CTCs to predict which patients would do well when treated with abiraterone. "We found that this had very good predictive ability in determining up front in patients whether or not abiraterone will work," asserted Dr. Miyamoto.

That was proof of principle, Dr. Miyamoto said. The next step is to try it with other drugs and replicate the results in larger cohorts. Dr. Miyamoto reported that in men with metastatic prostate cancer that had already spread mostly to the bone, the number of patients with a detectable CTC signal was low.

"In those few patients who had a high CTC score, there was actually a higher incidence of pathologic spread of cancer beyond the prostate found at the time of surgery," Dr. Miyamoto said. That suggests that CTC scoring could be used to predict ahead of time whether patients have early dissemination of disease and need more aggressive local therapies, or additional systemic therapies up front. GEN



A microfluidic device for the separation of CTCs has been developed by Sunitha Nagrath, Ph.D., and colleagues at the University of Michigan. The device, which is depicted in this schematic, incorporates a labyrinthine path that subjects small cells (such as healthy blood cells) and large cells (such as CTCs) to different forces, channeling the cells to different streamlines.

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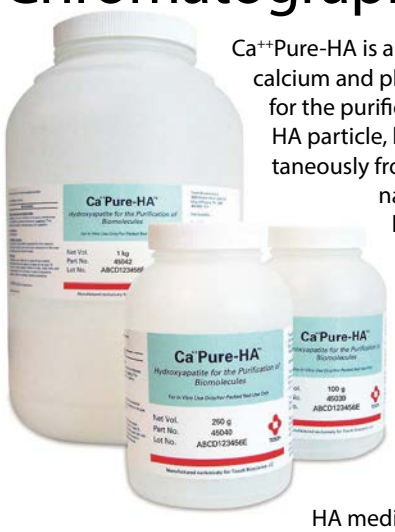

Ca^{++}Pure-HA is a hydroxyapatite chromatography media composed of calcium and phosphate and offers unique mixed mode properties for the purification of biomolecules. The formation of the Ca^{++}Pure-HA particle, both the ligand and the base bead, is created simultaneously from the same source of materials. Its highly selective nature often separates proteins otherwise shown to be homogeneous by electrophoresis and other chromatographic techniques. The highly selective and robust nature of Ca^{++}Pure-HA offers the flexibility to use this resin at any stage in a process from capture to final polishing. The media resin has been sintered at high temperatures for increased mechanical and pH stability, allowing it to withstand the rigors of industrial-scale applications. It has a demonstrated dynamic binding capacity (DBC), at 5% breakthrough, of 55 mg mAb/mg resin at 5 min residence time. Ca^{++}Pure-HA media is effective for the removal of dimer and higher-order aggregates from purified mAb samples. When it is operated using potassium phosphate buffer as a loading buffer and potassium chloride as an elution buffer, the aggregate content is reduced from 6.6% to as low as 1.3%.

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Multichannel Reagent Reservoirs

This popular reagent reservoir product family has expanded to include polypropylene reservoirs with enhanced chemical compatibility that perfectly complements the existing polystyrene reservoirs. Polypropylene Multichannel Reagent Reservoirs—available in 10, 25, and 100 mL sizes—offer all the advantages of their polystyrene counterparts. The disposable reservoir fits securely within a sturdy reusable base and features visible integrated volume graduations for ease of use, allowing rapid, accurate filling. The low dead volume and deep trough offer maximum fluid recovery, with pour-back spouts molded into the corners of each reagent reservoir to enable convenient, the spill-free return of unused reagent to the source container. The reservoirs can also act as lids, preventing evaporation and spillage. A space-saving, stackable design significantly reduces storage requirements, and there is less material waste than with other commercially available regular polypropylene reservoirs.

Integra Biosciences
www.integra-biosciences.com

NGS Panels

Due to the wide variety of genes that are interrogated in any given study, *de novo* targeted NGS has been hindered by the relative difficulty and high cost of custom panel design. To combat this, NEBNext Direct® Custom Ready Panels™ utilize a hybridization-based enrichment technology which allows users to quickly mix and match any combination of ~850 pre-synthesized gene "baits" associated with diseases such as cancer, autism, and cystic fibrosis. Unlike alternative hybridization methods, NEBNext Direct panels do not require upfront library preparation. The applications of the rapid, single-day protocol include rapid hybridization of both DNA strands with biotinylated probes, enzymatic removal of off-target sequences, identification of PCR duplicates with unique molecular tags, and high-throughput library pooling with barcodes.

New England BioLabs
www.neb.com

LCMS Ion Source

This new high-speed platform will significantly improve productivity in high-throughput laboratories performing toxicology, drug discovery, and food safety applications. It incorporates the LDTD (laser diode thermal desorption) ion source technology and is designed to synchronize with the speed of the Shimadzu LCMS-8060 triple quadrupole mass spectrometer. The platform ionization mode selector allows users to rapidly switch between liquid chromatography and LDTD technologies without moving or disconnecting any component of the mass spectrometer. With a simple click, the scientist can select the best way to develop, produce, screen and confirm their analysis.

Shimadzu Scientific Instruments
www.ssi.shimadzu.com

Mid-Scale Lyophilizer

The Genesis Lyophilizer freeze dryer has been designed to meet virtually any R&D or small-scale production lyophilization application. It is highly configurable and designed to deliver shelf temperatures as low as -70°C, condenser temperatures to -85°C, and operate in bulk or stoppering configurations. Allowing for easy and intuitive scale-up from research, the Genesis makes the perfect mid-scale lyophilizer. The compact, free-standing, mobile design enables the system to be optimally configured to almost any application. The product chamber, shelves and condenser chamber are made of durable 316L stainless steel, with a squared product chamber that ensures easy cleaning and maximum shelf area. A 4-inch diameter port increases vapor flow from the product to the condenser chamber maximizing system productivity. For applications where a sterile environment is critical, an easy-to-install clean room version of the Genesis lyophilizer is also available.

SP Scientific
www.spscientific.com

IGG Subclass Antibodies

Introducing a new series of affinity-purified, unconjugated, anti-human antibodies to the IgG subclasses, including IgG1, IgG2, IgG3, and IgG4. All of these polyclonal antibodies have been expressly designed for *in vitro* research use procedures as an integral component in solid-phase enzyme immunoassay based test methods, especially ELISA's, as well as in other potential testing applications. These antibodies are of benefit to the needs of biopharmaceutical, clinical, and life science researchers, along with other allied health professionals and IVD manufacturers. All of the antibodies exhibit both high levels of purity and lot-to-lot consistencies while demonstrating outstanding degrees of specificity. In addition, all feature a long shelf-life stability claim and are available in standard fill formats of 0.5 mg/mL unitized-sized vials.

The Binding Site
www.immunologicals.com

GEN Calendar

October

October 17–19
SLAS Advanced 3D Human Models and High-Content Analysis Conference to be held in Leiden, The Netherlands.
Web: www.slas.org/europe/events-in-europe/hcs-2018.

October 23–24
NGS & Clinical Diagnostics USA Congress to be held in Boston, MA.
Web: www.nextgenerationsequencingusa-congress.com.

October 24
Applied Pharmaceutical Nanotechnology to be held in Cambridge, MA.
Web: www.bostonsociety.org/APN.

October 24–25
CRISPR-Cas9 Technology and Genetic Engineering to be held in Billerica, MA.
Web: crisprcongress.conferenceseries.com.

October 24–26
Exosomes & Liquid Biopsies Europe to be held in Rotterdam, The Netherlands.
Web: selectbiosciences.com/conferences/index.aspx?conf=EXLBPEU2018.

October 24–26
Well Characterized Biologics & Biological Assays to be held in Rockville, MD. Web: lifesciences.knect365.com/well-characterized-biologicals.

October 25–26
Stem Cell Congress to be held in London, U.K. Web: www.stemcells-congress.com.

Meeting Highlight

November 1–3

AMP
to be held in San Antonio, TX.

The theme of this year's Association for Molecular Pathology (AMP) 2018 Annual Meeting & Expo is "Precision Medicine Starts Here." Network with molecular diagnostics professionals. Learn about and discuss medical treatments and technologies in precision medicine that begin on the molecular level.

Register Today
amp18.amp.org

November

November 3–7
Society for Neuroscience to be held in San Diego, CA.
Web: www.sfn.org/annual-meeting/neuroscience-2018.

November 5–7
Bio Europe to be held in Copenhagen, Denmark.
Web: ebdgroup.knect365.com/bioeurope.

November 8–9
Genome Editing Congress to be held in London, U.K.
Web: www.genomeediting-congress.com.

November 8–9
Next Generation Sequencing & Clinical Diagnostics Congress to be held in London, U.K.
Web: www.oxfordglobal.co.uk/nextgenerationsequencing-congress.

November 8–9
Single Cell Analysis Congress to be held in London, U.K.
Web: www.oxfordglobal.co.uk/singlecell-congress.

November 8–9
Synthetic Biology UK Congress to be held in London, U.K.
Web: www.oxfordglobal.co.uk/syntheticbiology-congress.

November 13–14
SLAS Americas Sample Management Symposium to be held in Boston, MA.
Web: www.slas.org/events/americas-2018-sample-management-symposium.

Send your **Upcoming Events** to calendar@GENengnews.com

Company Index

Profile

SPHERE FLUIDICS appointed **Tris Vaughan, Ph.D.**, as scientific director. Dr. Vaughan is vice-president, R&D antibody discovery and protein engineering, at MedImmune. Prior to joining that company, he was a postdoctoral fellow at the University of Toronto.

Profile

RELAY THERAPEUTICS appointed **Steven Kafka, Ph.D.**, to its board of directors. Dr. Kafka is the former president, COO, and CBO of Foundation Medicine. Prior to that, he held several positions at Aileron Therapeutics.

Profile

Joseph Leveque, M.D., joined **SYNTHORX** as CMO. Dr. Leveque is the former CMO of ARMO Biosciences (acquired by Eli Lilly). Before that, he was CMO of EMD Serono, and vice president and head of U.S. medical at Bristol-Myers Squibb. Earlier in his career, Dr. Leveque was vice president, medical and scientific affairs, at Onyx Pharmaceuticals.

AKOUOS established a scientific advisory board, consisting of: **Luk Vandenberghe, Ph.D.**; **Jean Bennett, M.D., Ph.D.**; **Michael McKenna, M.D.**; **William Sewell, Ph.D.**; **Richard Smith, M.D.**; and **Aaron Tward, M.D., Ph.D.**

BIOSYSMETRICS established a strategic advisory board, consisting of: **Calum MacRae, M.D., Ph.D.**; **Chinnappa Kodira**; **Paul Boutros, Ph.D.**; **Robert DeVita, Ph.D.**; and **Gene Salkind, M.D.**

OPTIBIOTIX hired **Sofia Kolyda, Ph.D.**, as director of R&D.

OWLSTONE MEDICAL expanded its scientific advisory board to include: **Patrick Bossuyt, Ph.D.**; **Christian Frezza, Ph.D.**; **Chris A. Mayhew, Ph.D.**; **Anil Modak, Ph.D.**; and **Alan Boobis, Ph.D.**

TORQUE welcomed **James Mullen** as chairman of its board of directors; **Maykin Ho, Ph.D.**, as strategic advisor; and **Mario Sznol, M.D.**, as a member of its scientific advisory board.

IGM BIOSCIENCES hired **Daniel S. Chen, M.D., Ph.D.**, as CMO, and appointed **William Strohl, Ph.D.**, to its board of directors.

SIRNAOMICS added **David Evans, Ph.D.**, as CSO.

Yan Wang, Ph.D., is the new CEO of **CYTOVANCE BIOLOGICS**.

PROVENTION BIO hired **Mark Rigby, M.D., Ph.D.**, as vice-president, clinical development.

ROCKWELL MEDICAL hired **Stuart Paul** as CEO.

Margo Roberts, Ph.D., joined **CELYAD's** board of directors and scientific committee.

ALKAHEST hired **Bruce Morimoto, Ph.D.**, as vice president, drug development and operations.

Chris Nowers is the new CEO and executive director of **CELL MEDICA**.

HALO LABS hired **Robert Wicke** as CEO.

Ignacio Faus joined **MOLOGEN** as CEO and member of its board.

ONCOBIOLOGICS promoted **Lawrence A. Kenyon** to president and CEO.

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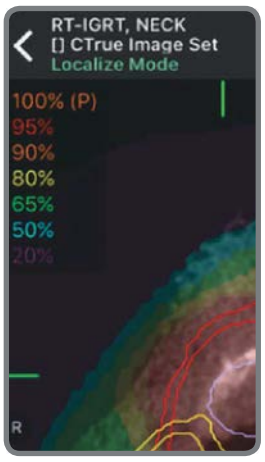
Advertiser Index

GEN Best Science Apps

Mobile MIM ★★★★

Platform: iPhone/iPad **Available for Android:** No **Cost:** Free



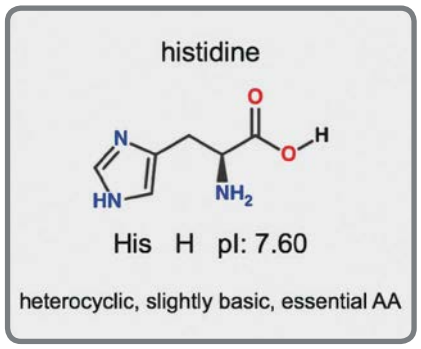
Descriptive user guide, protects patient information

Mobile MIM is a useful app for medical professionals who need to examine medical images from patients but find themselves without access to a full workstation. The app allows users to upload images from a variety of scans, including X-rays, PET scans, and CT scans. Users can then examine the images within the app, which allows for rotation, focusing, and annotation of the images. The app provides sample data so that users can get a better sense of the app and also provides a full user guide. Additionally, in order to protect sensitive patient data, the app encrypts all downloaded files once a passcode is set up by the user. Mobile MIM is a useful app for those in the medical field, particularly those that find themselves in need of analyzing patient data without access to typical means of examining images.

Amino Acids: Quiz & Flashcards ★★★★

Platform: iPhone/iPad **Available for Android:** No **Cost:** $0.99

Easy to use, users can choose to review the structures or quiz themselves

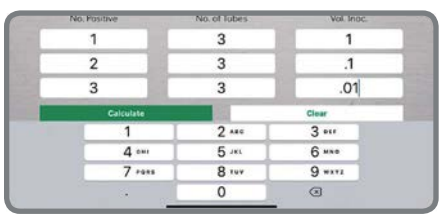


Memorizing the names and structures of common amino acids is the bane of any biochemistry student's existence. Luckily, apps like Amino Acids: Quiz & Flashcards exist to make the task easier. The app provides a number of different ways for learning and reviewing the common amino acids and their structures. Users can quiz themselves based on the amino acid structure alone or by utilizing the multiple-choice feature, in which users have to choose the correct structure based on the name of the amino acid given to them. Users can also choose to time themselves to see if they can beat the clock while identifying the structures. Additionally, there are flashcards available for users who just want to flip through and review the names and structures of the amino acids. Amino Acids: Quiz & Flashcards is a useful app for biochemistry students or for anyone who finds that they need some review of the topic.

MPN Calculator ★★★

Platform: iPhone/iPad **Available for Android:** No **Cost:** Free

Straightforward and easy to use *Users can't save their calculations in the app*



The MPN Calculator is a useful tool for biologists who need to estimate how many microorganisms they have in a sample. The app is fairly basic but is straightforward and easy to use for its designed purpose. After making serial dilutions of their samples, users can input into the app the number of tubes that are positive for the organism, the total number of tubes, and the volume inoculated within each tube. With this information, the app utilizes probability formulas to estimate the likely average density of microorganisms that are contained in the sample. In addition to providing the mean density, the app also gives users a 95% confidence interval of its calculation. The app includes detailed instructions on how to use it in order to get the best results and the app is well-referenced. The MPN Calculator is a useful tool for biologists at the bench who need to determine the density of organisms in their samples.

Papers 3 ★★★

Platform: iPhone/iPad **Available for Android:** No **Cost:** Free app, but requires subscription for full use

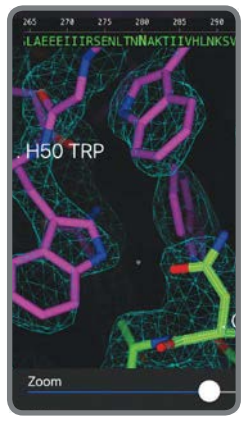
Easy to search for publications and customize collections of papers *Steep subscription cost, downloading publications takes up significant storage*

As a scientist, keeping up with scientific literature is essential, but keeping your library of publications organized can be difficult. Enter Papers, an app that does all the work for you. The app makes it easy to search for, save, and organize the scientific literature that you need. Users can search for publications directly within the app using an advanced number of search criteria. Once you have found the publication that you are looking for you can save the paper to your own library. Libraries can be organized into collections, with the option of making folders and subfolders that users can sort their papers into for easy retrieval. Additionally, users can share their collections with others, making collaboration easier than ever. Although the app itself is free, users are only allowed a 30-day free trial before they have to pay for full access to the app, which runs from $50–80. However, the Papers app is definitely worth trying out, if only to see how much more organized your library of publications can be.

HIV Antibody Database ★★★

Platform: iPhone/iPad **Available for Android:** No **Cost:** Free

Large catalog of antibodies, detailed information *Somewhat difficult to navigate*



Although treatment of HIV has come a long way in the last few decades, researchers are still actively trying to find better ways of combating this deadly virus. To help with this goal, there are apps like the HIV Antibody Database, which compiles into one place an extensive list of antibodies that recognize and neutralize HIV-1. The app allows users to browse through antibodies, their structures, and viruses. The app includes extensive information about each antibody catalogued in the app, including coverage curves, IC50 tables, and crystal data. Users can also display the structure of the antibodies and are able to examine each part of the structure in detail. The app also includes references so that users can turn to the primary literature for additional information. The HIV Antibody Database is an excellent resource for scientists studying HIV and searching for more information about the antibodies that can combat it.

Lab Assist: The Lab Companion ★★

Platform: iPhone/iPad **Available for Android:** No **Cost:** Free

Easy to use, beautiful app design *Disruptive pop-up ads*

Lab Assist: The Lab Companion combines the utilities of lab apps that typically stand alone into one, well-designed format. The app contains three main functionalities that are all useful to any scientist at the bench: a timer, a counter, and a dilution calculator. The counter allows users to count up for two separate samples and to assign a name to each, which is useful for avoiding confusion. The dilution calculator allows users to input values so that they can calculate mass concentration, molar concentration, and percentage concentration. The app interface is beautifully designed and easy to navigate and use. The one major downside is that there are a lot of pop-up ads that can be disruptive if you are trying to use the app. However, Lab Assist: The Lab Companion is still a convenient tool that combines the functionality of several useful lab apps into one place.

All of the links to the URLs described above are posted on *GEN's* website, www.GENengnews.com.
To suggest an app for Best Science Apps, please send the URL to: jsterling@GENengnews.com.

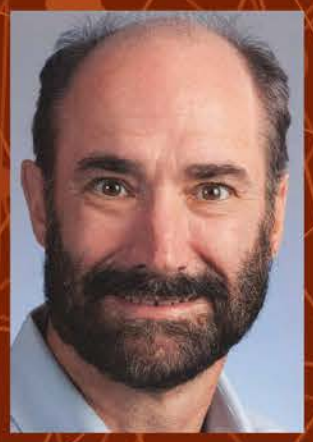
Key: Strong Points, Weak Points. Ratings: Excellent ★★★★, Very Good ★★★, Good ★★

Clinical OMICs
Molecular Diagnostics in Personalized Medicine

Webinars

How Multi-Omics Profiling Can Redefine Precision Health and Medicine

Speaker



Michael Snyder, Ph.D.
Professor and Chair of the Department of Genetics
Director of the Center for Genomics and Personalized Medicine
Stanford University School of Medicine

Thursday
October 11, 2018

8:00 am PT
11:00 am ET
17:00 CET

DURATION: 45 minutes
COST: Complimentary

For Research Use Only. Not for use in diagnostic procedures.

Free Registration!

www.clinicalomics.com/iPOP

Detailed information for generating an average cellular metabolic profile could be as useful and common for physicians as taking a patient's vital signs. Rich datasets that reflect a comprehensive clinical research compendium of biological, physiological, and molecular signatures will reveal insights into the factors that contribute to health or disease over an individual's lifetime. Coupling relatively routine datasets such as genomes and transcriptomes with newer types—most notably mass spectrometry-based proteomes, and metabolomes produces an "integrative Personal Omics Profile," (iPOP). Join us for this exciting Clinical OMICS webinar where we will discuss how this multi-omics approach, when combined with clinical research observation, can unlock the complexity of systems biology at the individual level. Some key areas that will be discussed:

- High-resolution biochemical profiles using metabolomics and mass spectrometry as part of iPOP
- The potential of iPOP demonstrated in diabetes risk
- Recent examples of iPOP showing the potential to identify individuals what will manifest signs of illness
- The future of the iPOP approach

A live Q&A session will follow the presentation, offering you a chance to pose questions to our expert panelist.

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