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Ob(nox)ious fibrosis

By Benjamin Boettner, Associate Editor

Although Esbriet pirfenidone, the only marketed drug for idiopathic pulmonary fibrosis, has shown a mortality benefit, the effects are modest and there remains room for improvement. New preclinical findings suggest that inhibiting NADPH oxidase 4 could both resolve and reverse fibrosis.[1]

Based on the results, **Genkyotex S.A.** plans to advance its GKT137831 NADPH oxidase 4 (NOX4) inhibitor to treat IPF.

IPF, like other forms of fibrosis, is the consequence of a failed epithelial repair process triggered by inflammation or injury. The process begins when transforming growth factor β1 (TGFB1) activates myofibroblasts.

Normally, this allows for the production of extracellular matrix components like collagen that serve as a substrate for epithelial repair. But in patients with IPF, activated myofibroblasts overproliferate and produce excessive amounts of extracellular matrix components.

The result is fibrotic scars that prevent re-epithelialization and ultimately render organ systems dysfunctional.[2]

InterMune Inc.'s Esbriet pirfenidone is approved in multiple countries. The drug is a small molecule inhibitor of proinflammatory cytokines and profibrotic cytokines that targets p38 mitogen-activated protein kinase (p38 MAPK; MAPK14).

The next drug likely to be marketed for IPF is Vargatef nintedanib from **Boehringer Ingelheim GmbH**. Vargatef is a small molecule inhibitor of multiple proangiogenic kinases that is in Phase III testing for IPF. The compound significantly slowed declines in forced vital capacity; however, pooled Phase III data show that it produced only nonsignificant improvements in all-cause mortality.

IPF's incidence and prevalence increase with age—most patients are more than 65 years old at the time of diagnosis.

Now, a team led by **The University of Alabama at Birmingham** researchers Louise Hecker and Victor Thannickal thinks NOX4 may be at the root of IPF's age association.

Thannickal is a professor of medicine and pathology and director of the Division of Pulmonary, Allergy and Critical Care at The University of Alabama at Birmingham. Hecker recently moved to **The University of Arizona**, where she is an assistant professor of medicine. The study was coauthored by former Genkyotex CSO Eric Meldrum.

The group already had clues that the target was involved in IPF. In 2009, they showed that *NOX4* expression was stimulated in fibroblastic foci by TGFB1 in a mouse model of IPF and in samples from patients with IPF. siRNAs targeting *Nox4* were able to prevent fibrogenesis.[3]

In the new study, the group compared chemically induced lung fibrosis in young and aged mice. Although both sets of animals developed fibrosis, only the young animals were able to resolve the fibrotic lesions.

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Additional studies pointed to Nox4-generated oxidative stress as the key difference between the young and old animals. Fibrotic areas in old but not young mice were characterized by an imbalance between two redox systems: profibrotic superoxide-producing Nox4 and anti-fibrotic nuclear factor (erythroid-derived 2)-like 2 (Nfe2l2; Nrf2).

NRF2 is a transcriptional regulator that activates a battery of genes that cooperate to reduce reactive oxygen species.

Intranasal delivery of a *Nox4*-specific siRNA reduced senescence markers and eliminated apoptosis resistance—both of which are associated with fibrosis in aged animals—and restored the ability to resolve fibrosis. The researchers saw similar results when they used GKT137831.

The University of Alabama team found the NRF2 response to elevated superoxide levels to be impeded specifically in fibrotic cells of aged mice.

Accordingly, sulforaphane, an activator of Nrf2, was able to resensitize lung myofibroblasts isolated from aged, injured mice to apoptosis. This could potentially reduce collagen-producing cell numbers in fibrotic foci.

Data were published in *Science Translational Medicine*.

"The data provide clear evidence of the fundamental role of NOX4 in lung fibrosis in a model which best reflects the disease in patients. It points the way to the use of a NOX4 inhibitor for the treatment of IPF," said Genkyotex CEO Ursula Ney.

GKT137831 is a dual inhibitor of NOX4 and NOX1. The molecule is in Phase II testing to treat diabetic nephropathy.

Both Ney and Yves Gorin think that inhibiting NOX4 will be a better option than stimulating NRF2 in fibrotic disease. "The targeted inhibition of NOX4 is likely to be safer than the chronic activation of hundreds of NRF2-responsive genes," said Ney.

Gorin is an associate professor of medicine at **The University of Texas Health Science Center at San Antonio** whose work is focused on redox systems including NOX4 in different diseases.

Resolving the mechanisms

Scott Turner, EVP of R&D at **KineMed Inc.**, wanted to see more evidence that Nox4 inhibition helps to actively resolve fibrosis in aged mice.

"Clearly, direct evidence of reduced collagen production or increased degradation would be useful in defining the *in vivo* effects of the inhibitor, as would be a demonstration of an influx of resolving cell types and increased matrix metalloproteinase activity," he said.

KineMed is developing biomarkers and assays, including dynamic measurements of collagen in fibrotic contexts.

"Clearly, direct evidence of reduced collagen production or increased degradation would be useful in defining the *in vivo* effects of the inhibitor, as would be a demonstration of an influx of resolving cell types and increased matrix metalloproteinase activity."
—Scott Turner, KineMed Inc.

Gorin said that it would be useful to identify the molecular mechanisms in young mice that keep a healthy balance of Nox4- and Nrf2-dependent biological responses.

Gorin and Bruno Crestani think that NOX4 inhibitors could have utility beyond IPF because myofibroblasts are activated in virtually every fibrotic organ.

Crestani is a professor at **Paris Diderot University**, at **Institut National de la Santé et de la Recherche Médicale** (INSERM) unit 1152 and in the pneumology department and Centre for Rare Lung Diseases at the **Hospital Bichat**.

"It would be interesting to also study the effect of NOX4 inhibition and the modulation of NOX4/NRF2 coupling on fibrosis-associated complications in the kidney or the heart in elderly diabetes patients," said Gorin.

Paul Higgins, a professor and director at **Albany Medical College**, agreed. "Other models of experimental fibrosis particularly in the heart and renal systems should be evaluated with respect to age," he said. Higgins did caution that the precise NOX proteins involved in each tissue, however, might differ.

Thannickal told *SciBX* that he has applied for funding from the **NIH**'s Centers for Advanced Diagnostics and Experimental Therapeutics program to develop additional NOX4 inhibitors. He said that he is looking for industry partners.

The University of Alabama at Birmingham has a patent covering NOX4-specific siRNAs that block fibrotic processes.

Genkyotex owns IP covering GKT137831's composition of matter.

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COMPANIES AND INSTITUTIONS MENTIONED

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Institut National de la Santé et de la Recherche Médicale, Paris, France
InterMune Inc. (NASDAQ:ITMN), Brisbane, Calif.
KineMed Inc., Emeryville, Calif.
National Institutes of Health, Bethesda, Md.
Paris Diderot University, Paris, France
The University of Alabama at Birmingham, Birmingham, Ala.
The University of Arizona, Tucson, Ariz.
The University of Texas Health Science Center at San Antonio, San Antonio, Texas

Translational tidbits

By Lev Osherovich and Kai-Jye Lou, Senior Writers

Berg's big data dive

Berg Pharma LLC is seeking to validate its Interrogative Biology toolkit by striking academic partnerships with the **University of Miami Miller School of Medicine** and **Medical University of South Carolina**. Berg will use tissue banks and patient data from the medical schools to discover biomarkers and targets for cancer and cardiovascular, CNS, metabolic and autoimmune diseases.

The platform combines a patient's molecular data with clinical records and demographic data. The goal of the systems biology approach is to help doctors select treatment regimens and help insurance companies develop formularies.

The key, said Berg president, cofounder and CTO Niven Narain, is getting access. "We want to partner with leading laboratories with exceptional access to tissue samples and databases," he said.

The University of Miami deal gives Berg access to tissue samples and clinical data from hundreds of patients with a variety of illnesses. Berg will perform genomic, metabolomic and proteomic analyses; crunch the data in search of predictive biomarkers and potential targets; and work with clinical researchers to validate interesting hits.

Narain said that the partnership broadens an existing deal with the laboratory of Pascal Goldschmidt, dean and SVP for medical affairs at the Miller School of Medicine. That project has yielded a prognostic marker for heart failure that Narain expects will enter clinical testing in 18–24 months.

Narain noted that Berg is also collaborating with the University of Miami to test the university's dendrimer-based drug delivery technology in preclinical disease models..

The Medical University of South Carolina deal is narrower. It is focused on discovering markers for lupus in African-American women, a high-risk but understudied patient population.

Berg will analyze samples and clinical records from a collection maintained by professor of medicine and microbiology Gary Gilkeson.

"We have a very well-characterized chronological database of patients with matched kidney biopsies," said Gilkeson. "We have a large African-American cohort in this group. A lot of research genetics has focused on Asians and Caucasians, but we don't know much about the causes of lupus in African Americans."

He said that the study with Berg initially will include 70 patients and 70 controls matched for demographics. "We have serum and urine samples.

Table 1. Selected public-private partnerships for April 2014. In April, two new consortia—PDE4NPD and SYMPATH—received a total of about €13.8 million ($19 million) from the EU's Seventh Framework Programme, and **The Scripps Research Institute** launched **Scripps Advance LLC** to house assets and IP related to discrete early stage research projects.
Source: BioCentury archives

Companies	Institutions	Business area	Disclosed value	Purpose
Diaxonhit (Euronext:ALEHT); **InnaVirVax S.A.**	**Institut National de la Santé et de la Recherche Médicale** (INSERM)	Infectious disease; diagnostics	€8 million ($11.1 million)	PROTHEVIH project to develop HIV vaccine VAC-3S, companion diagnostic test CO-3S and prognostic test DIAG-3S
Iota Pharmaceuticals Ltd.; **Top Institute Pharma**; **European ScreeningPort**	**Oswaldo Cruz Foundation**; **Spanish National Research Council**; **Theodor Bilharz Research Institute**; **University of Antwerp**; **University of Glasgow**; **University of Kent**; **VU University Amsterdam**; **European Commission**	Infectious disease	€7.8 million ($10.7 million)	PDE4NPD consortium to discover new molecules with drug-like properties against parasitic phosphodiesterases
Roslin Cells Ltd.	**Cell Therapy Catapult**; **Irish Blood Transfusion Service**; **Loughborough University**; **NHS Blood and Transplant**; **Scottish National Blood Transfusion Service**; University of Glasgow; **The University of Edinburgh**; **Wellcome Trust**	Hematology	£5 million ($8.4 million)	BloodPharma consortium to generate red blood cells from pluripotent stem cells for transfusions
Affiris AG; **Biolution GmbH**; **Prosenex Ambulatorium Betriebs GmbH**	**French Clinical Research Infrastructure Network**; **Juelich Research Center GmbH**; **Medical University Innsbruck**; **University Hospital Bordeaux**; **University Hospital Toulouse**; European Commission	Neurology	€6 million ($8.3 million)	SYMPATH consortium to develop two vaccine candidates from Affiris against Parkinson's disease (PD) and multiple system atrophy (MSA)
UCB Group (Euronext:UCB)	**Weill Cornell Medical College**	Musculoskeletal disease; Endocrine/ metabolic disease; genomics	$8 million	Advance the college's translational research programs in bone disorders, metabolic disease and rare genetic variant analysis
Berg Pharma LLC	**University of Miami Miller School of Medicine**	Cancer; neurology; cardiovascular disease; endocrine/ metabolic disease	Undisclosed	Discover and develop treatments for cancer and CNS, cardiovascular and metabolic diseases

(Continues on p. 5)

Table 1. Selected public-private partnerships for April 2014. (continued)

Companies	Institutions	Business area	Disclosed value	Purpose
Daiichi Sankyo Co. Ltd. (Tokyo:4568)	**University of California, San Francisco**	Neurology; diagnostics	Undisclosed	Discover and develop new drugs and diagnostics for multiple neurodegenerative diseases
Eli Lilly and Co. (NYSE:LLY)	**Unitio**	Endocrine/ metabolic disease	Unavailable	Use real-world, patient-centric data to identify ways to improve care and advance outcomes in type 1 diabetes
Epigenetix Inc.	**Neomed Institute**	Cancer	Undisclosed	Develop bromodomain containing 4 (BRD4) inhibitors as epigenetic modulators to treat cancer
Ferring Pharmaceuticals A/S; **Merck & Co. Inc.** (NYSE:MRK)	**World Health Organization**	Hematology	Unavailable	Develop a new formulation of Ferring's carbetocin to treat postpartum hemorrhage that is designed to be stable at room temperature
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)	**The University of Texas MD Anderson Cancer Center**	Cancer	Undisclosed	Develop immunotherapies for cancer under MD Anderson's Moon Shots Program
Johnson & Johnson (NYSE:JNJ); Scripps Advance	Scripps Research Institute	Other	Undisclosed	Identify and invest in early stage biomedical research projects
Merck KGaA (Xetra:MRK); **Pfizer Inc.** (NYSE:PFE)	**Broad Institute of MIT and Harvard**	Autoimmune disease	Unavailable	Identify biomarkers to better define target patient populations for systemic lupus erythematosus (SLE) and lupus nephritis
Sinovac Biotech Ltd. (NASDAQ:SVA)	**Institute for Translational Vaccinology**; World Health Organization	Infectious disease	Undisclosed	Develop Sabin inactivated polio vaccine under the public-private Global Polio Eradication Initiative

We will provide the samples to Berg, and they will then run this on their data platform, putting the results into diagrams," said Gilkeson. "Once we get the results, we will need to confirm them using other samples and to follow up on the pathways to see if they make biologic sense. We can test some of these ideas in animal models or against data sets from other demographic groups."

Gilkeson said that the combination of genetic and biochemical analyses offered by Berg will provide a comprehensive picture of lupus progression.

"Putting all that together has never been done before, mainly because nobody has the money," said Gilkeson. "You can't get funding from NIH for a non–hypothesis driven fishing expedition like this."

In both deals, Berg has right of first negotiation to license new IP and will share resulting royalties with its academic partners.

Closing the gap with iPS cells

Researchers at **The New York Stem Cell Foundation** (NYSCF) have reported a key technical achievement that brings somatic cell nuclear transfer (SCNT) technologies closer in line with those used to generate induced pluripotent stem (iPS) cells.[1] The new approach could enable the translation of SCNT technologies to the cell-replacement field.

SCNT is used to generate embryonic stem cells (ESCs), but the technology has lagged behind the technologies used to generate iPS cells. The latter are generally more scalable and subject to fewer funding restrictions and ethical considerations.

However, one area in which SCNT could have an edge over iPS cell technologies is in developing cell-replacement therapies. Cells derived from ESCs should have fewer safety risks than those derived from iPS cells because the reprogramming factors used to generate iPS cells have the potential to introduce genetic abnormalities.

In a study published in *Nature*, a group led by Dieter Egli has now published an SCNT-based protocol for generating ESC lines that worked with fibroblasts from a 32-year-old female with type 1 diabetes. The resulting ESC lines could be differentiated into insulin-producing pancreatic cells as well as neuronal cells.

"Because the biology of human oocytes is poorly understood, and protocols established in animals could not be directly translated to human oocytes, the derivation of stem cells [with SCNT] using adult somatic cells is a very large technical step," said Egli, a senior research fellow at the NYSCF. "With these results, we are now more on par with that which has been achieved with iPS cells."

Other groups have published SCNT-based protocols for generating ESC lines from fetal human somatic cells[2,3] and more recently with somatic cells from a healthy adult.[4] However, Egli said that using somatic cells from an adult with a disease that could be treated with an organ or tissue transplant is a key technical step toward realizing potential therapeutic applications with the technology.

> **"With these results, we are now more on par with that which has been achieved with iPS cells."**
>
> ***—Dieter Egli, The New York Stem Cell Foundation***

Islet and pancreas transplantation are both treatment options for patients with type 1 diabetes.

"This research is about making stem cells from patients who are alive today. The results provide proof of principle that we can make cell types of interest in a disease—in this case, type 1 diabetes—from an adult patient's skin sample for potential use in therapeutic cell replacement therapies," he told *SciBX*.

Egli said that his group is now running studies to compare genetically identical pluripotent human stem cells generated via SCNT and iPS cell

(Continues on p. 6)

Antibiotic team building

By Chris Cain, Senior Writer

The **National Institute of Allergy and Infectious Diseases** has awarded a 5-year, $26 million grant to a center for antibiotic development that brings together researchers from **Rutgers University**, **The Rockefeller University** and **Cubist Pharmaceuticals Inc.** The goal is to take discovery-stage programs through lead optimization and preclinical development to a partnering event.

The award marks the 13th Center for Excellence in Translational Research (CETR) funded by the National Institute of Allergy and Infectious Diseases (NIAID) in 2014.

The center will be led by David Perlin, executive director of and a professor at **The Public Health Research Institute at Rutgers University**. He told *SciBX* that the regulatory hurdles and financial disincentives to antibiotic development have stymied industry interest in early stage projects—a gap the center hopes to bridge.

Indeed, antibiotic developers and infectious disease specialists have repeatedly told *SciBX* that there is a dearth of new antibiotics in the pipeline capable of dealing with emerging drug-resistant bacterial threats.[1,2]

"There is a big gulf to cross from fundamental discovery and identification of small molecule inhibitors to refining and validating antibacterial compounds," Perlin said. "Companies have not been as patient to go through this early process—they essentially want molecules ready to go into preclinical assays. By having this center, we can advance molecules to that point."

The funding will support projects from five labs as well as their use of associated core facilities. These include a medicinal chemistry core with a structural biology subcore to optimize molecules; a pharmacokinetic and toxicology profiling core; an *in vitro* screening core to assess compound potency and spectrum of activity; and an animal model core that provides disease models for skin and soft tissue infections and systemic and pulmonary infections, and that permits an analysis of markers of disease progression.

The center has biosafety level 3 capabilities and access to a regional biocontainment laboratory that can handle multidrug-resistant pathogens including *Mycobacterium tuberculosis*, *Bacillus anthracis* and *Yersinia pestis*.

Perlin said that Rutgers is well equipped in part because it has been a member of the NIAID-funded Northeast Biodefense Center. Regional Centers for Excellence are consortiums that have been funded by large NIAID grants since 2003, although funding for the program expires this year. The CETR is a follow-on NIAID program.

The academic research teams participating in the CETR are led by Sean Brady, an associate professor at Rockefeller; David Alland, associate dean for clinical research and a professor of medicine at **Rutgers New Jersey Medical School**; Joel Freundlich, an assistant professor of pharmacology and physiology and medicine at the medical school; and Richard Ebright, a professor of chemistry and chemical biology at Rutgers University and lab director at the **Waksman Institute of Microbiology at Rutgers University**. Cubist is the lone industry member.

Mechanism scattershot

Perlin told *SciBX* that the projects being developed by the center include a mix of chemical classes that hit validated targets as well as new antibiotic mechanisms of action.

For example, Brady's lab will be developing compounds sourced from peptidic libraries derived from environmental samples of bacteria. He has published extensively on using sequencing, computational methods and *in vitro* analysis to identify new antimicrobial natural products.

(Continues on p. 7)

(Continued from "**Translational tidbits**," p. 5)

reprogramming to understand differences between the two types of cells. His group also is investigating strategies to improve the transplantation of islet β cells and block the autoimmune response against pancreatic cells.

NYSCF has filed a patent application covering the work described in *Nature*. The licensing status is undisclosed.

Public-private partnership roundup

April was a relatively quiet month for public-private partnerships, with the bulk of the activity occurring outside the U.S. Two new consortia—PDE4NPD and SYMPATH—received a total of about €13.8 million ($19 million) from the EU's Seventh Framework Programme. Stateside, **The Scripps Research Institute** launched Scripps Advance LLC to house assets and IP related to discrete early stage research projects and attract pharma investment in translational assets.[5] Scripps Advance's first pharma partner is **Johnson & Johnson** (*see* **Table 1, "Selected public-private partnerships for April 2014"**).

Osherovich, L. & Lou, K.-J. *SciBX* **7**(20); doi:10.1038/scibx.2014.574
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COMPANIES AND INSTITUTIONS MENTIONED

Berg Pharma LLC, Farmingham, Mass.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
Medical University of South Carolina, Charleston, S.C.
The New York Stem Cell Foundation, New York, N.Y.
Scripps Advance LLC, Jupiter, Fla.
The Scripps Research Institute, La Jolla, Calif.
University of Miami Miller School of Medicine, Miami, Fla.

A second project will be focused on compounds that inhibit mycolic acid synthesis, a key component of the cell wall in mycobacteria including *M. tuberculosis*. A third will work on developing Bayesian modeling to accelerate antibacterial discovery.

Ebright told *SciBX* that his lab will pursue a project focused on optimizing new arylpropionyl-phloroglucinol–based inhibitors of bacterial RNA polymerase (RNAP). The inhibitors bind to a site on the protein that is distinct from that of existing inhibitors such as rifampicin. The project is one of many in his lab that is investigating drug discovery against RNAP.

In a separate, non-CETR project published last month in *eLife*, Ebright used extensive crystallography and other *in vitro* studies to show that the natural product antibiotic GE23077 binds to yet another site on RNAP that is distinct from that bound by rifampicin.[3]

His team further showed that bipartite inhibitors linking GE23077 to rifampicin had activity against strains resistant to either compound alone. Patent applications covering the bipartite compounds have been filed and are available for licensing. Composition-of-matter patents covering GE23077 are owned by **Naicons s.r.l.**

Cubist input

The fifth and final project in the center comes from Cubist, which inherited its role in the CETR through its 2013 acquisition of Trius Therapeutics Inc. The deal's most visible asset was tedizolid, an oxazolidinone that is under FDA review to treat acute bacterial skin and skin structure infections (ABSSSI). However, Cubist also gained Trius' DNA gyrase inhibitors, which were included as part of the CETR application.

Cubist's EVP of R&D and CSO Steven Gilman told *SciBX* that the gyrase program caught the company's attention at an early stage. "One of the things we looked at in Trius when we were starting to talk with them about a relationship was the DNA gyrase program. We had been interested in DNA gyrase for a long time—quinolones had been very useful drugs for many years with a good spectrum of activity against Gram-positive and Gram-negative bacteria, and most of the problems with resistance are related to the quinolone structure. This program could offer quinolone-like efficacy with a scaffold that is distinct."

Gilman acknowledged that Cubist has significantly more in-house resources than Trius and thus may not rely as heavily on the center's chemistry capabilities as Trius might have.

Thus, he said, "the benefit of this consortium is more of an intellectual exchange—the researchers are well-known scientists with whom we'd like to collaborate more deeply. We bring years and years of biopharmaceutical industry experience to this consortium. Cubist has a wealth of experience, understands what companies are looking for, and can help groups create a package of information that is strong and comprehensive and could open up additional funding and partnerships."

Perlin agreed and said that he had originally specifically sought out Trius before it was acquired to join the CETR to get industry input on projects. He added that the DNA gyrase inhibitor program was further along than other projects, and its inclusion helped balance the stage of development of projects at the center and improve their chances of accelerating a program into the clinic.

Perlin added that the regulatory environment for antibiotic development is improving. "There is money to be made with anti-infectives; we know there is a need for them, and companies will in fact get back into it," he said.

Indeed, in the past six months **Roche** has entered into at least three antibiotic discovery partnerships with biotechs.[4]

Although this CETR is focused on antibiotics, the larger NIAID CETR program is casting a broad net for anti-infective strategies. Two centers established so far this year include a $32 million project to study the role of autophagy in pathogen-host defense with researchers from the **Washington University in St. Louis School of Medicine**, **Massachusetts General Hospital**, the **Broad Institute of MIT and Harvard** and **The University of Texas Southwestern Medical Center**.

The other center involves 15 institutions and received a $28 million grant to develop Ebola treatments.

NIAID intends to commit $75 million to the CETR program in FY2014 and to fund a total of 10–20 awards.

Cain, C. *SciBX* 7(20); doi:10.1038/scibx.2014.575
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Naicons s.r.l., Milan, Italy
National Institute of Allergy and Infectious Diseases, Bethesda, Md.
The Public Health Research Institute at Rutgers University, Newark, N.J.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
The Rockefeller University, New York, N.Y.
Rutgers New Jersey Medical School, Newark, N.J.
Rutgers University, Newark, N.J.
The University of Texas Southwestern Medical Center, Dallas, Texas
Waksman Institute of Microbiology at Rutgers University, Piscataway, N.J.
Washington University in St. Louis School of Medicine, St. Louis, Mo.

A big heart

By Tracey Baas, Senior Editor

Cell therapies in the clinic for heart failure draw from divergent sources of nonembryonic stem cells but have shown only minimal improvements in cardiac function. A **University of Washington** team thinks human embryonic stem cell–derived cardiomyocytes may do a better job and has used the cells to remuscularize infarcted monkey hearts.[1]

Larger preclinical studies, using more consistent methods, will need to address the arrhythmia seen in the study and show conclusively that the cells improve cardiac function.

At least 11 companies have stem cell therapies in Phase II testing or earlier to treat myocardial infarction (MI). The clinical-stage products use either autologous or allogeneic adult stem cells. Those cells are rare in mature tissues, and as a result isolation and expansion is a challenge.

Mesenchymal stem cells (MSCs) are a more plentiful source of adult stem cells and can differentiate into osteoblasts, chondrocytes and adipocytes. *Ex vivo*, MSCs can be induced to differentiate into cardiomyocytes. However, the flexibility in their differentiation capacity could be a drawback, as MSCs could result in unwanted cell types forming in cardiac tissue.

Alternatively, human embryonic stem cells (hESCs) can be grown easily in culture and can differentiate into a single cell type, such as cardiomyocytes. The problem is quantity—protocols to provide large-scale quantities for cardiac repair need to be optimized.

The University of Washington team previously transplanted about 10^6, 10^7 and 10^8 hESC-cardiomyocytes (CMs) that were cultured and directly transferred to mouse, rat and guinea pig models of MI.[2–5] For a nonhuman primate model, the researchers estimated that about 10^9 cells would be required for a 50 g macaque heart, which was a big hurdle technically. These numbers would be close to what is required for a 300 g human heart.

To produce enough cells for the nonhuman primate model, the group differentiated hESCs into cardiomyocytes. The resulting cells spontaneously beat in culture. Rather than transferring the cells directly into an animal after culture, the cells were treated with a prosurvival cocktail and then were cryopreserved.

Two weeks before hESC-CM delivery, pigtail macaques underwent myocardial ischemia/reperfusion to generate infarcts. Five days before cell delivery, they were put on an immunosuppressive regimen to prevent graft rejection.

Four animals received cells via epicardial puncture sites that were stabilized with sutures. Two animals underwent the same procedure with vehicle.

The treated monkeys showed remuscularization of the infarct area with grafts averaging about 40% of the infarct mass. From week 2 to week 12, the engrafted cells progressively matured, as shown by increased myofibril alignment, sarcomere registration and cardiomyocyte diameter.

Because of the small number of animals used, the team could not conclude whether the procedure significantly improved cardiac function. Two of the four treated animals showed increases in ejection fraction, whereas the other two did not.

Overall, the hESC-CM grafts showed infiltration of lymphocytes and perfusion of blood vessels. The grafts were visible by imaging and showed electromechanical coupling to macaque hearts.

Continuous electrocardiogram recording showed that the four treated monkeys had periods of arrhythmia during the first two weeks of recovery. The authors wrote that further studies are needed to tease out the underlying mechanisms that contributed to arrhythmia.

Results were published in *Nature*. The authors did not return requests for comment.

Eduardo Marbán, director of the **Cedars-Sinai Heart Institute**, noted that because the researchers were focused on showing the feasibility of the approach, "the protocol varied significantly from animal to animal. An important next step would be to adhere to a single experimental protocol in a rigorous manner that would enable statistical analysis once all the data are collected."

> **"An important next step would be to adhere to a single experimental protocol in a rigorous manner that would enable statistical analysis once all the data are collected."**
>
> ***—Eduardo Marbán, Cedars-Sinai Heart Institute***

Marbán also was the principal investigator on the NIH-funded Phase I CADUCEUS (CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction) trial of cardiosphere-derived autologous stem cells for heart regeneration after MI. His technology, licensed by **Capricor Therapeutics Inc.**, forms the basis for an ongoing Phase I/II trial, ALLSTAR (ALLogeneic heart Stem cells To Achieve myocardial Regeneration), which uses allogeneic, cardiosphere-derived stem cells for the same indication.

In the CADUCEUS trial, 17 patients received the cell-based therapy and had no cardiac tumors or major adverse cardiac events. Patients showed a decrease in cardiac scar mass and an increase in viable heart mass and contractility; no such changes were seen in eight patients randomly assigned to receive standard care. But there were no substantial differences in left ventricular ejection fraction between the two groups.

Early steps

The researchers now need to generate more evidence of increased cardiac function, show that arrhythmias can be circumvented or are not problematic and rule out graft rejection and teratoma formation.

Roberto Bolli, director of the Division of Cardiology and the Institute of Molecular Cardiology and vice chair for research in the Department of Medicine at the **University of Louisville**, said that the study authors were jumping the gun with their conclusions that "large hESC-CM grafts are successfully perfused by host vasculature and are viable long term."

"The results are preliminary and anecdotal. Four monkeys were given cells. For the researchers to conclude that their data show that the grafts 'are viable long term' is extremely premature," he said. "With a follow-up of 2 weeks for 1 monkey, 4 weeks for 2 monkeys and 12 weeks for 1 monkey, no such conclusion is possible."

Bolli wants to see larger and more detailed studies, with a longer follow-up, that show functional relevance and safety. "We do not know whether transplantation of these cells improved cardiac function or the structure of the heart, which are both major goals of cell therapy," he said.

Thus, Bolli is not convinced that the embryonic cells are an advance over the adult stem cell–derived products in the clinic.

Bolli said that he was still concerned about the potential of ESCs to form tumors and induce graft rejection. In contrast, he said, "to date, not one single major adverse effect has been reported that could be ascribed to transplantation of adult stem cells in patients."

The key safety issue thus far is arrhythmias. Moreover, humans have larger and slower hearts than macaques—two factors that correlate with increased risk of developing arrhythmias after cell engraftment.[6–8]

Mary Wagner, an assistant professor of pediatrics and director of the Center for Cardiovascular Biology at the **Emory University School of Medicine**, said that mapping the electrical activity of the heart would be a good way to evaluate if arrhythmias are caused by the transplanted hESC-CMs or represent a more general response to the procedure. "For example, 3D mapping techniques for quantifying the propagation of electrical activity could map the arrhythmias and lead to a better understanding of whether cellular engraftment contributes to the side effect," she said.

"A lack of gap junctions early after transplantation in immature cardiomyocytes may produce poor coupling between the host and graft and can result in slow or intermittent conduction and possibly arrhythmia," said Chunhui Xu, an associate professor of pediatrics and director of the Cardiomyocyte Stem Cell Laboratory at the Emory University School of Medicine. "In the *Nature* study, at 14 days post-transplantation, there is little evidence of gap junction protein α1, 43 kDa (GJA1; CX43; connexin-43)—suggesting cardiomyocyte immaturity—but [the protein] is seen 12 weeks post-transplantation."

Xu said that techniques that speed up maturation of engrafted cardiomyocytes could help prevent arrhythmias.

"An alternative strategy could be to introduce earlier cardiomyocytes, or cardiac progenitors, before they have begun to autonomously beat," said Emile Nuwaysir, COO and VP of R&D, manufacturing and quality systems at **Cellular Dynamics International Inc.** "This may work because the cells, as they mature *in vivo*, might listen to the endogenous pacemaker as they become electromechanically active and would have no adaptation period after injection."

Markus Krane, head of the Department of Experimental Surgery at the **German Heart Centre Munich**, said that the cell therapy could benefit from a different delivery approach. "In an aged person with a chronically failing heart and increased epicardial fat, simple repetitive cardiac puncture would be a difficult clinical scenario," he said. "In this setting, it would be more advantageous to deliver small, preformed hESC-CM-based cell clusters or tissue grafts for implantation under the epicardial fat layer."

Philippe Menasche liked the idea of optimizing stem cell delivery by a pericardial flap. Menasche is a professor of thoracic and cardiovascular surgery at the **University Paris Descartes**, chief of the Heart Failure Surgery Unit at **Georges Pompidou European Hospital** and director of an **Institut National de la Santé et de la Recherche Médicale** (INSERM) laboratory focused on cell therapy for cardiovascular diseases.

Menasche also is a co-investigator on a Phase I trial at **Paris Public Hospital**. The ESCORT (transplantation of human Embryonic Stem Cell–derived prOgenitors in severe heaRT failure) trial is recruiting patients.

"The pericardial flap is considered a growth factor–rich tissue, so using it to cover hESC-CMs or a scaffold seeded with hESC-CMs could potentially provide the cells with a natural, tissue-based, prosurvival environment," he said.

"By using a contractile patch made from hESC-CMs or human induced pluripotent stem cell–derived CMs—potentially sutured over the most damaged cardiac region—this would provide a way to enhance the pumping function of the damaged cardiac tissue. The transplanted patch could also be insulated from the host's own cardiac cells," added Jonathan Epstein. "This insulation could block the arrhythmia that is induced by competing electromechanical coupling of two sets of cardiomyocytes—self and nonself."

Epstein is chair of the Department of Cell and Developmental Biology and scientific director of the Penn Cardiovascular Institute at the **Perelman School of Medicine at the University of Pennsylvania**.

The patent and licensing status of the University of Washington's findings was not available at publication time.

Baas, T. *SciBX* 7(20); doi:10.1038/scibx.2014.576
Published online May 22, 2014

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COMPANIES AND INSTITUTIONS MENTIONED

Capricor Therapeutics Inc. (OTCQB:CAPR), Beverly Hills, Calif.
Cedars-Sinai Heart Institute, Los Angeles, Calif.
Cellular Dynamics International Inc. (NASDAQ:ICEL), Madison, Wis.
Emory University School of Medicine, Atlanta, Ga.
Georges Pompidou European Hospital, Paris, France
German Heart Centre Munich, Munich, Germany
Institut National de la Santé et de la Recherche Médicale, Paris, France
Paris Public Hospital, Paris, France
Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pa.
University of Louisville, Louisville, Ky.
University of Washington, Seattle, Wash.
University Paris Descartes, Paris, France

This week in therapeutics

THE DISTILLERY brings you this week's most essential scientific findings in therapeutics, distilled by *SciBX* editors from a weekly review of more than 400 papers in 41 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

This week in therapeutics includes important research findings on targets and compounds, grouped first by disease class and then alphabetically by indication.

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer				
Cancer	Integrin $\alpha_5\beta_3$ (CD49e/CD61)	*In vitro* and mouse studies suggest inhibiting CD49e/CD61 could help treat tumors resistant to receptor tyrosine kinase (RTK) inhibitors. Cultured lung, breast and pancreatic cancer cell lines expressing CD49e/CD61 had a survival advantage over nonexpressing cells and showed greater resistance to RTK inhibitors such as Tarceva erlotinib. In tumor biopsies from patients whose disease had progressed on Tarceva, CD49e/CD61 expression was higher than that in samples taken prior to treatment or from untreated patients. In mice with subcutaneous lung and prostate tumors, Tarceva plus the proteasome inhibitor Velcade bortezomib, which blocks a target downstream of CD49e/CD61, decreased tumor volume compared with Tarceva alone. Next steps include testing the combination in patients who have progressed on Tarceva alone. Astellas Pharma Inc., Chugai Pharmaceutical Co. Ltd. and the Genentech Inc. unit of Roche market Tarceva to treat pancreatic cancer and non–small cell lung cancer (NSCLC). Takeda Pharmaceutical Co. Ltd. and Johnson & Johnson market Velcade to treat multiple myeloma (MM) and mantle cell lymphoma (MCL). ***SciBX* 7(20); doi:10.1038/scibx.2014.577** **Published online May 22, 2014**	Patent applications filed covering use of NF-κB pathway inhibitors to treat tumors resistant to RTK inhibitors; unlicensed	Seguin, L. *et al. Nat. Cell Biol.*; published online April 20, 2014; doi:10.1038/ncb2953 **Contact:** David A. Cheresh, University of California, San Diego, La Jolla, Calif. e-mail: dcheresh@ucsd.edu
Cancer	Notch 1 (NOTCH1); estrogen receptor-β	*In vitro* and mouse studies suggest estrogen receptor-β agonists could help treat squamous cell carcinoma (SCC). In an analysis of published datasets, *NOTCH1* and its transcriptional activators, including estrogen receptor-β, were downregulated in SCC cells but not in keratinocytes. In skin, oral and lung SCCs, virus vector–mediated estrogen receptor-β overexpression or a selective small molecule estrogen receptor-β agonist increased NOTCH1 expression and decreased cell proliferation compared with no alteration or with vehicle. In mice intradermally injected with human head and neck SCC cells, an estrogen receptor-β agonist decreased tumor size and proliferation compared with vehicle. Next steps include identifying additional agonists. Bionovo Inc. has the estrogen receptor-β agonist MF101 in Phase III testing to treat menopause. At least five other companies have estrogen receptor-β agonists in Phase II or earlier testing. ***SciBX* 7(20); doi:10.1038/scibx.2014.578** **Published online May 22, 2014**	Provisional patent application filed; available for licensing	Brooks, Y.S. *et al. J. Clin. Invest.*; published online April 17, 2014; doi:10.1172/JCI72718 **Contact:** G. Paolo Dotto, University of Lausanne, Lausanne, Switzerland e-mail: paolo.dotto@unil.ch

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cardiovascular disease				
Restenosis	Pyruvate dehydrogenase kinase 2 (PDK2)	Rodent, rabbit and porcine studies suggest inhibiting PDK2 in coronary artery transplants could prevent restenosis. In rodent and rabbit models of injury-induced arterial restenosis, shRNA against *PDK2* or the PDK2 inhibitor dichloroacetate (DCA) decreased markers of restenosis such as myointimal proliferation compared with control shRNA or vehicle. In a swine model of coronary artery restenosis, DCA decreased graft restenosis compared with vehicle. Next steps include investigating DCA's long-term safety and defining a patient population most likely to benefit from the molecule. ***SciBX* 7(20); doi:10.1038/scibx.2014.579** **Published online May 22, 2014**	Patent application filed; licensing details available from Stanford University's Office of Technology Licensing	Deuse, T. *et al. Nature*; published online April 20, 2014; doi:10.1038/nature13232 **Contact:** Sonja Schrepfer, Stanford University, Stanford, Calif. e-mail: schrepfer@stanford.edu
Hematology				
Thrombocytopenia	Chemokine CXC motif ligand 12 (CXCL12; SDF-1)	Mouse studies suggest SDF-1 could help treat thrombocytopenia. In mice, i.v. SDF-1 or stabilization of endogenous SDF-1 with a dipeptidyl peptidase-4 (DPP-4; CD26) inhibitor increased migration of platelet-producing megakaryocytes to the vasculature and increased circulating platelet levels compared with vehicle. In irradiated mice, thrombopoietin plus SDF-1 increased circulating platelet levels compared with either treatment alone. Next steps include studying the long-term effects of SDF-1 on megakaryocytes. ***SciBX* 7(20); doi:10.1038/scibx.2014.580** **Published online May 22, 2014**	Unpatented; license status not applicable	Niswander, L.M. *et al. Blood*; published online April 15, 2014; doi:10.1182/blood-2014-01-547638 **Contact:** James Palis, University of Rochester Medical Center, Rochester, N.Y. e-mail: james_palis@urmc.rochester.edu
Infectious disease				
Bacterial infection	Bacterial RNA polymerase (RNAP)	*In vitro* studies identified bivalent inhibitors of RNAP that could help treat bacterial infections. Biochemical and crystallography studies showed that the cyclic-peptide antibiotic GE23077 inhibits bacterial growth by binding the active site of RNAP, a previously unknown site for antibacterial action. A new antibiotic was synthesized by linking GE23077 to rifamycin—which inhibits RNAP activity by binding a distinct site on the protein—and this dual-acting compound inhibited the growth of bacteria resistant to either parent compound. Next steps include optimizing derivatives (*see* **Antibiotic team building, page 6**). ***SciBX* 7(20); doi:10.1038/scibx.2014.581** **Published online May 22, 2014**	Patent application filed; available for licensing	Zhang, Y. *et al. eLife*; published online April 22, 2014; doi:10.7554/eLife.02450 **Contact:** Richard H. Ebright, Rutgers University, Piscataway, N.J. e-mail: ebright@waksman.rutgers.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Respiratory syncytial virus (RSV)	RSV F protein	Cell culture and mouse studies suggest nanoparticle-formulated, double-stapled RSV F peptides could be used to prevent RSV infection. In a human cell line infected with RSV, pretreatment with a double-stapled RSV F peptide decreased virus infection and virus-cell fusion events compared with vehicle pretreatment. In a mouse model of RSV infection, nasal pretreatment with the peptide decreased upper and lower respiratory infection compared with vehicle pretreatment. Also in the mouse model, intratracheal pretreatment with the peptide led to significant decreases in lower respiratory infection ($p<0.01$). Next steps could include advancing the prophylaxis to the clinic. ***SciBX* 7(20); doi:10.1038/scibx.2014.582** **Published online May 22, 2014**	Patented; available for licensing	Bird, G.H. *et al. J. Clin. Invest.*; published online April 17, 2014; doi:10.1172/JCI71856 **Contact:** Loren D. Walensky, Dana-Farber Cancer Institute, Boston, Mass. e-mail: loren_walensky@dfci.harvard.edu **Contact:** Shyam S. Mohapatra, James A. Haley Veterans' Hospital, Tampa, Fla. e-mail: shyam.mohapatra@va.gov
Neurology				
Addiction	Metabotropic glutamate receptor subtype 2 (mGluR2; GRM2); mGluR3 (GRM3)	*In vitro* and rat studies suggest positive allosteric modulators (PAMs) of both mGluR2 and mGluR3 could help treat cocaine addiction. In *in vitro* receptor binding assays, the optimal PAM potentiated binding of glutamate to mGluR2 with an EC_{50} value of 0.14 μM and to mGluR3 with an EC_{50} value of 0.3 μM. In a rat model of cocaine dependence, the PAM decreased cocaine self-administration compared with vehicle. Next steps include optimization studies and generation of selective activators of mGluR3. Addex Therapeutics Ltd. and Johnson & Johnson have the mGluR2 PAM ADX71149 (JNJ-40411813) in Phase II trials to treat anxiety and schizophrenia. ***SciBX* 7(20); doi:10.1038/scibx.2014.583** **Published online May 22, 2014**	Patent application filed; available for licensing	Dhanya, R.-P. *et al. J. Med. Chem.*; published online April 15, 2014; doi:10.1021/jm5000563 **Contact:** Nicholas D.P. Cosford, Sanford-Burnham Medical Research Institute, La Jolla, Calif. e-mail: ncosford@sanfordburnham.org
Addiction	Neurotrophic tyrosine kinase receptor 2 (NTRK2; TrkB)	Rat studies suggest stimulating TrkB could help treat drug addiction. In a rat model of cocaine addiction, systemic pretreatment with the TrkB agonist 7,8-dihydroxyflavone suppressed cocaine-induced conditional place preference, whereas vehicle did not. Next steps include elucidating the mechanisms connecting TrkB and NMDA receptor NR2B subtype (GRIN2B; NR2B) activation and identifying specific GRIN2B agonists. ***SciBX* 7(20); doi:10.1038/scibx.2014.584** **Published online May 22, 2014**	Unpatented; licensing status not applicable	Otis, J.M. *et al. J. Neurosci.*; published online April 23, 2014; doi:10.1523/JNEUROSCI.4980-13.2014 **Contact:** Devin Mueller, University of Wisconsin–Milwaukee, Milwaukee, Wis. e-mail: devinm@uwm.edu
Alzheimer's disease (AD)	CREB-regulated transcription coactivator 1 (CRTC1; TORC1)	Human and mouse studies suggest agonizing CRTC1 could help treat AD. In patients with intermediate and advanced AD pathology, hippocampal levels of CRTC1 were lower than those of healthy control subjects. In a transgenic mouse model of AD, hippocampal Crtc1 activation was lower than that in normal mice. In the transgenic model, hippocampal injection of an adeno-associated viral (AAV) vector encoding mouse *Crtc1* decreased AD-associated learning and memory deficits compared with injection of a control vector encoding *GFP*. Next steps could include identifying brain-penetrant CRTC1 agonists. ***SciBX* 7(20); doi:10.1038/scibx.2014.585** **Published online May 22, 2014**	Patent and licensing status unavailable	Parra-Damas, A. *et al. J. Neurosci.*; published online April 23, 2014; doi:10.1523/JNEUROSCI.5288-13.2014 **Contact:** Carlos A. Saura, Autonomous University of Barcelona, Barcelona, Spain e-mail: carlos.saura@uab.es

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Alzheimer's disease (AD)	Prostaglandin E_2 receptor EP4 subtype (prostanoid EP4 receptor; PTGER4)	Studies in mice and human samples suggest agonizing PTGER4 could help treat AD. In mouse microglia cultured with β-amyloid 42, a Ptger4 agonist suppressed expression of inflammatory genes. In a mouse model of AD, monocyte-specific *Ptger4* knockout increased expression of inflammatory genes and β-amyloid deposition in the brain compared with no alteration. Cortical section samples from patients with AD or mild cognitive impairment (MCI) had lower PTGER4 expression than samples from healthy individuals. Next steps could include testing Ptger4 activation in animal models of AD. ***SciBX* 7(20); doi:10.1038/scibx.2014.586** **Published online May 22, 2014**	Patent and licensing status unavailable	Woodling, N.S. *et al. J. Neurosci.*; published online April 23, 2014; doi:10.1523/JNEUROSCI.0410-14.2014 **Contact:** Katrin I. Andreasson, Stanford University School of Medicine, Stanford, Calif. e-mail: kandreas@stanford.edu
Alzheimer's disease (AD)	Vacuolar protein sorting 35 homolog (VPS35); VPS29; amyloid precursor protein (APP)	*In silico* and *in vitro* studies identified a pharmacological retromer chaperone that inhibits pathogenic processing of APP and could help treat AD. *In silico* docking and screening identified small molecules that bind the retromer complex at the interface of its VPS35 and VPS29 subunits. In *in vitro* biochemical assays, one compound bound to and stabilized the complex. In cultured murine hippocampal neurons, the compound increased expression of various retromer subunits and decreased accumulation of pathogenic APP derivatives compared with vehicle. Next steps include identifying drug-like retromer chaperones that cross the blood brain barrier and evaluating their safety. ***SciBX* 7(20); doi:10.1038/scibx.2014.587** **Published online May 22, 2014**	Patent applications filed; licensed to an undisclosed newco; available for partnering	Mecozzi, V.J. *et al. Nat. Chem. Biol.*; published online April 20, 2014; doi:10.1038/nchembio.1508 **Contact:** Scott A. Small, Columbia University College of Physicians and Surgeons, New York, N.Y. e-mail: sas68@columbia.edu **Contact:** Dagmar Ringe, Brandeis University, Waltham, Mass. e-mail: ringe@brandeis.edu **Contact:** Gregory A. Petsko, Weill Cornell Medical College, New York, N.Y. e-mail: gpetsko@med.cornell.edu
Depression	Hyperpolarization activated cyclic nucleotide-gated potassium channel 2 (HCN2)	Mouse studies suggest increasing hyperpolarization-activated current in dopaminergic neurons in the brain's ventral tegmental area (VTA) could help protect against depression. Earlier mouse studies showed that social defeat stress enhanced hyperpolarization-activated current in VTA neurons. Mice resilient to social defeat stress had a hyperpolarization-activated current that was stronger than that in mice susceptible to such stress. In the susceptible mice, VTA dopaminergic neuron–specific overexpression of HCN2 to further potentiate the hyperpolarization-activated current reversed social avoidance and other depression-associated behaviors. Next steps include studies to understand the molecular mechanisms that underlie the stronger hyperpolarization-activated current in depression-resilient mice. ***SciBX* 7(20); doi:10.1038/scibx.2014.588** **Published online May 22, 2014**	Unpatented; licensing status not applicable	Friedman, A.K. *et al. Science*; published online April 18, 2014; doi:10.1126/science.1249240 **Contact:** Ming-Hu Han, Icahn School of Medicine at Mount Sinai, New York, N.Y. e-mail: ming-hu.han@mssm.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Depression; psychosis	Serotonin (5-HT_6) receptor; serotonin (5-HT_7) receptor; dopamine D2 receptor	*In vitro* and rat studies suggest inhibitors of 5-HT_6, 5-HT_7 and dopamine D2 receptors could help treat psychosis and depression in patients with dementia. Chemical synthesis of arylsulfonamine-benzoxazole analogs identified several compounds as selective nanomolar inhibitors of the three receptors. In two rat models of drug-induced hyperactivity, one lead compound decreased hyperlocomotive activity compared with vehicle without also causing sedation or memory impairment. In a rat model of forced swim–induced depression, the lead compound decreased immobility compared with vehicle. Ongoing preclinical work by Adamed Group includes developing the lead compound, AD-N03, to treat depression and psychosis. ***SciBX* 7(20); doi:10.1038/scibx.2014.589** **Published online May 22, 2014**	Patented by Adamed; available for licensing	Kolaczkowski, M. *et al. J. Med. Chem.*; published online May 7, 2014; doi:10.1021/jm401895u **Contact:** Marcin Kolaczkowski, Adamed Group, Czosnow, Poland e-mail: marcin.kolaczkowski@adamed.com.pl
Neuroinflammation	Colony-stimulating factor 1 receptor (CSF1R; C-FMS; CD115)	Mouse studies suggest CSF1R inhibition could help suppress microglia-mediated neuroinflammation. In mice, systemic treatment with the CSF1R inhibitor PLX3397 decreased brain microglia by 50% after 3 days and by 99% after 21 days without causing deficits in learning, memory, motor function or behavior. In the PLX3397-treated, microglia-depleted mice, brain-resident progenitor cells appeared 3 days after stopping PLX3397 and microglia numbers reverted to wild-type levels 21 days after stopping treatment. Next steps include investigating whether CSF1R inhibition could block microglia activation after traumatic brain injury or in neuroinflammatory diseases, including Alzheimer's disease (AD). Daiichi Sankyo Co. Ltd. has PLX3397 in Phase II testing to treat various cancers. ***SciBX* 7(20); doi:10.1038/scibx.2014.590** **Published online May 22, 2014**	Patent on PLX3397 held by Daiichi Sankyo; licensing status unavailable	Elmore, M.R.P. *et al. Neuron*; published online April 16, 2014; doi:10.1016/j.neuron.2014.02.040 **Contact:** Kim N. Green, University of California, Irvine, Calif. e-mail: kngreen@uci.edu
Neurology	Cleavage and polyadenylation factor 1 subunit 1 (CLP1)	Studies in patients and mice identified a mutation in *CLP1* that causes CNS dysfunction. A homozygous R140H recessive missense mutation in the gene encoding the tRNA splicing kinase CLP1 was identified in affected individuals from five families with similar CNS symptoms, including microcephaly and motor sensory defects. *In vitro*, expression of the mutation decreased kinase activity, binding of CLP1 to the tRNA splicing endonuclease complex and tRNA splicing compared with wild-type gene expression. In mice, expression of a kinase-dead mutant decreased cortical brain volume compared with wild-type kinase expression and caused microcephaly and cortical neuron cell death. Next steps include identifying the mechanism linking *CLP1* mutation to motor neuron death. ***SciBX* 7(20); doi:10.1038/scibx.2014.591** **Published online May 22, 2014**	Findings unpatented; available for licensing	Karaca, E. *et al. Cell*; published online April 24, 2014; doi:10.1016/j.cell.2014.02.058 **Contact:** James R. Lupski, Baylor College of Medicine, Houston, Texas e-mail: jlupski@bcm.edu **Contact:** Josef M. Penninger, Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna, Austria e-mail: josef.penninger@imba.oeaw.ac.at **Contact:** Javier Martinez, same affiliation as above e-mail: javier.martinez@imba.oeaw.ac.at

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Neurology	Cleavage and polyadenylation factor 1 subunit 1 (CLP1)	Studies in patients and zebrafish suggest increasing *CLP1* expression could help treat neurodegenerative conditions caused by *CLP1* mutations. Exome sequencing of 2,000 families with children who had neurological disorders identified the loss-of-function R140H mutation in *CLP1* in 4 independent Turkish families. Zebrafish engineered with the *Clp1* mutation had neurodegeneration and neuromotor defects. In the engineered zebrafish, injection of wild-type human *CLP1* mRNA rescued some of the neuromotor defects, whereas injection of mutant human *CLP1* mRNA did not. Next steps could include developing strategies to increase expression of wild-type *CLP1* in human cells carrying the R140H mutation. ***SciBX*** **7(20); doi:10.1038/scibx.2014.592** **Published online May 22, 2014**	Patent and licensing status unavailable	Schaffer, A.E. *et al. Cell*; published online April 24, 2014; doi:10.1016/j.cell.2014.03.049 **Contact:** Joseph G. Gleeson, University of California, San Diego, La Jolla, Calif. e-mail: jogleeson@ucsd.edu
Pain	Chemokine CX3C motif receptor 1 (CX3CR1); chemokine CX3C motif ligand 1 (CX3CL1; fractalkine)	*In vitro* and mouse studies suggest antagonizing CX3CR1 or preventing cleavage of CX3CL1 could help treat chemotherapy-associated neuropathic pain. In mice with vincristine-associated allodynia, knocking out *Cxrc31* blocked monocyte infiltration into the sciatic nerve, which is associated with increased pain sensitivity. In cultured, Cx3cr1^{+} monocytes, Cx3cl1 induced production of reactive oxygen species (ROS), which activate pain sensory neurons. In the cultured monocytes, knocking out *Cx3cr1* prevented Cx3cl1-induced ROS production. Next steps include testing the analgesic efficacy of CX3CR1 antagonists. ***SciBX*** **7(20); doi:10.1038/scibx.2014.593** **Published online May 22, 2014**	Unpatented; licensing status unavailable	Old, E.A. *et al. J. Clin. Invest.*; published online April 17, 2014; doi:10.1172/JCI71389 **Contact:** Marzia Malcangio, King's College London, London, U.K. e-mail: marzia.malcangio@kcl.ac.uk

This week in techniques

THE DISTILLERY brings you this week's most essential scientific findings in techniques, distilled by *SciBX* editors from a weekly review of more than 400 papers in 41 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

This week in techniques includes findings about research tools, disease models and manufacturing processes that have the potential to enable or improve all stages of drug discovery and development.

Approach	Summary	Licensing status	Publication and contact information
Assays & screens			
Assay to detect circulating methylated DNA (cMethDNA) to detect and monitor breast cancer	*In vitro* studies suggest a cMethDNA assay could help detect and monitor tumor burden. The cMethDNA assay amplifies and measures methylation of 10 genes with low methylation in normal cells but high methylation in breast cancer cells. The assay detected cancer with 96.4% specificity and 91.7% sensitivity in a set of 24 cancer and 28 normal samples, and results were validated in an additional 33 cancer and 27 normal samples. In samples from patients who responded to chemotherapy, cMethDNA levels were lower than those in samples from patients who had progressive disease. Next steps include validating clinical utility to monitor response to chemotherapy over time. ***SciBX* 7(20); doi:10.1038/scibx.2014.594** **Published online May 22, 2014**	Covered by issued and filed patents; available for licensing	Fackler, M.J. *et al. Cancer Res.*; published online April 15, 2014; doi:10.1158/0008-5472.CAN-13-3392 **Contact:** Saraswati Sukumar, The Sidney Kimmel Comprehensive Cancer Center at The Johns Hopkins University School of Medicine, Baltimore, Md. e-mail: saras@jhmi.edu
Census-based, whole-exome sequencing of circulating tumor cells (CTCs)	Census-based, whole-exome sequencing of CTCs could be used for blood-based diagnosis and monitoring of cancer metastasis. Census-based sequencing involves sequencing multiple DNA libraries generated from the same sample of CTCs and using variants present in more than one library to distinguish somatic mutations from sequencing errors. In 19 CTC libraries from a patient with metastatic castration-resistant prostate cancer (CRPC), census-based sequencing detected 73 somatic single-nucleotide variants (SSNVs), with 51 of them also detected in the primary tumor. In the same patient, among 93 SSVNs found in a sample from a lymph node metastasis, 47 were also detected in CTCs. Next steps include optimizing the method to decrease cost and increase throughput. ***SciBX* 7(20); doi:10.1038/scibx.2014.595** **Published online May 22, 2014**	Patent and licensing status unavailable	Lohr, J.G. *et al. Nat. Biotechnol.*; published online April 20, 2014; doi:10.1038/nbt.2892 **Contact:** J. Christopher Love, Broad Institute of MIT and Harvard, Cambridge, Mass. e-mail: clove@mit.edu **Contact:** Jesse S. Boehm, same affiliation as above e-mail: boehm@broadinstitute.org **Contact:** Gad Getz, same affiliation as above e-mail: gadgetz@broadinstitute.org
Nano-plasmonic exosome (nPLEX) sensor for high throughput, label-free, quantitative analysis of exosome proteins	Patient sample studies suggest an nPLEX sensor can help diagnose and/or monitor cancer treatment. The nPLEX sensor is comprised of an array of nanopores in a metal film functionalized with affinity ligands for specific proteins and a miniaturized imaging system. Binding of affinity ligands to proteins on exosomes results in a spectral shift. In ascites derived from patients with ovarian cancer, epithelial cell adhesion molecule (EpCAM) and CD24 arrays detected more exosomes and higher levels of protein per exosome than what was seen in ascites derived from patients with cirrhosis. Next steps include performing large-scale proteomic analysis on exosomes. ***SciBX* 7(20); doi:10.1038/scibx.2014.596** **Published online May 22, 2014**	Patents on nPLEX device and assays filed; available for licensing from Massachusetts General Hospital **Contact:** Dan Castro, Massachusetts General Hospital, Boston, Mass. e-mail: dc654@partners.org	Im, H. *et al. Nat. Biotechnol.*; published online April 20, 2014; doi:10.1038/nbt.2886 **Contact:** Hakho Lee, Massachusetts General Hospital, Boston, Mass. e-mail: hlee@mgh.harvard.edu **Contact:** Ralph Weissleder, same affiliation as above e-mail: rweissleder@mgh.harvard.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Chemistry			
Total synthesis of polyene compounds with *N*-methyliminodiacetic acid (MIDA) boronate building blocks	A method for total synthesis of polyene natural products could be useful for exploring their therapeutic applications. Analysis of a natural product database showed that over 75% of polyene compounds contain no more than 12 different polyene motifs. A two-step iterative coupling reaction used MIDA boronate analogs of polyene motifs to generate polyene chains up to 20 carbons long and utilized additional MIDA boronate analogs to add desired structures to the chain termini. Total synthesis with the method yielded milligram quantities of the antimicrobial and anticancer compound asnipyrone B and two other natural products. Next steps include automating the method and extending it to other classes of small molecules. ***SciBX* 7(20); doi:10.1038/scibx.2014.597** **Published online May 22, 2014**	Unpatented; licensing status not applicable	Woerly, E.M. *et al. Nat. Chem.*; published online May 11, 2014; doi:10.1038/nchem.1947 **Contact:** Martin D. Burke, University of Illinois at Urbana-Champaign, Urbana, Ill. e-mail: burke@scs.uiuc.edu
Drug platforms			
Cells with reduced N-glycan variability for therapeutic protein production	Cells with a modified Golgi N-glycosylation pathway could be useful for producing proteins with improved therapeutic properties. The GlycoDelete platform shortens the Golgi N-glycosylation pathway in mammalian cells to decrease N-glycan variability in proteins. In engineered human embryonic kidney (HEK) cells producing granulocyte macrophage colony-stimulating factor (GM-CSF; CSF2), the protein had activity comparable to that of GM-CSF produced in the same cell line without the GlycoDelete modifications. In GlycoDelete-engineered HEK cells producing an anti-CD20 IgG, the antibody had a lower clearance rate than anti-CD20 IgG generated in a cell line without the GlycoDelete modifications. Next steps could include using the GlycoDelete platform to generate additional therapeutic proteins and comparing their properties with the parent compound. ***SciBX* 7(20); doi:10.1038/scibx.2014.598** **Published online May 22, 2014**	Patent application filed; licensing status unavailable	Meuris, L. *et al. Nat. Biotechnol.*; published online April 20, 2014; doi:10.1038/nbt.2885 **Contact:** Nico Callewaert, Flanders Institute for Biotechnology (VIB), Ghent, Belgium e-mail: nico.callewaert@irc.vib-ugent.be
Uracil enrichment in the seed region of single guide RNAs (sgRNAs) decreases off-target binding by the clustered, regularly interspaced short palindromic repeats (CRISPR)-Cas9 platform	Cell culture studies suggest sgRNA sequence composition influences Cas9 binding specificity. In mouse embryonic stem cells stably expressing Cas9 and transfected with sgRNAs, ChIP-seq experiments revealed thousands of binding sites for the Cas9 protein. In the cells, genome editing was detected at 4 of 4 on-target sites and only 1 of 295 potential off-target sites for 4 sgRNAs. In the cells, sgRNAs with uracil-rich seed regions or downstream regions were expressed at lower levels and bound to fewer off-target sites than sgRNAs without uracil enrichment at the sites. Next steps could include establishing guidelines for engineering uracil-rich sgRNAs to improve editing-site selectivity. ***SciBX* 7(20); doi:10.1038/scibx.2014.599** **Published online May 22, 2014**	Patent and licensing status unavailable	Wu, X. *et al. Nat. Biotechnol.*; published online April 20, 2014; doi:10.1038/nbt.2889 **Contact:** Phillip A. Sharp, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: sharppa@mit.edu **Contact:** Feng Zhang, Broad Institute of MIT and Harvard, Cambridge, Mass. e-mail: zhang@broadinstitute.org
Imaging			
Monitoring oxygen levels in hypoxic tissues with a solid MRI contrast agent	A solid, oxygen-sensitive MRI contrast agent could help monitor treatment responses in hypoxic tissues and tumors. The agent consists of an oxygen-sensitive siloxane embedded in an inert siloxane polymer matrix. In rats subjected to variable amounts of oxygen, intramuscular injection of the agent enabled measurement of changing oxygen levels with MRI for at least four weeks without degrading. In a rat model of hindlimb ischemia, the agent enabled measurement of tissue oxygen levels, and its performance was unaffected by changes in blood flow, animal movements or physical pressure on the affected limb. Next steps include testing the safety of the agent. ***SciBX* 7(20); doi:10.1038/scibx.2014.600** **Published online May 22, 2014**	Patent application filed; available for licensing	Liu, V.H. *et al. Proc. Natl. Acad. Sci. USA*; published online April 21, 2014; doi:10.1073/pnas.1400015111 **Contact:** Michael J. Cima, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: mjcima@mit.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Markers			
Creatine riboside and *N*-acetylneuraminic acid (NANA) levels in urine as biomarkers of non–small cell lung cancer (NSCLC)	Patient studies suggest creatine riboside and NANA levels in urine could help diagnose or predict prognosis in NSCLC. In urine samples from 469 patients with NSCLC and 536 healthy individuals, creatine riboside and NANA were elevated in patient samples, with higher levels associated with worse prognosis. Results were validated in urisne samples from an additional cohort of 158 recently diagnosed patients with NSCLC. Next steps include validating the biomarkers in prospective studies in additional patients with lung cancer. *SciBX* 7(20); doi:10.1038/scibx.2014.601 Published online May 22, 2014	Patent application filed; available for licensing	Mathe, E.A. *et al. Cancer Res.*; published online April 15, 2014; doi:10.1158/0008-5472.CAN-14-0109 **Contact:** Curtis C. Harris, National Institutes of Health, Bethesda, Md. e-mail: curtis_harris@nih.gov

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