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SciBX: Science-Business eXchange will not be published on Dec. 27, 2012 and Jan. 3, 2013. It will resume its schedule the week of Jan. 7, 2013.

Translating mRNA vaccines

By *Chris Cain, Senior Writer*

A collaboration between **CureVac GmbH** and the **Friedrich Loeffler Institute** has produced *in vivo* evidence that an mRNA-based vaccine can prevent influenza A infection.¹ The results provide proof of concept for the company's vaccine platform in infectious disease, and **Sanofi** has options to the technology.

Conventional methods for influenza vaccine production rely on culturing the virus in chicken eggs or more recently in mammalian cells, followed by antigen purification. However, these approaches have multiple limitations, including a risk of contamination and an inability to rapidly upscale supply in response to an outbreak of a new strain.^{2,3}

To overcome these limitations and simplify production, groups have sought to engineer nucleic acid-based influenza vaccines. However, DNA-based vaccines have proven to be poorly or inconsistently immunogenic in humans.⁴

CureVac CEO Ingmar Hoerr told *SciBX* that this presented an opportunity for the company's mRNA-vaccine platform, which has induced immune responses in clinical trials of the company's therapeutic cancer vaccines. "In a prostate cancer Phase I/IIa study we saw T cell and antibody responses to our mRNA vaccine," he said.

In 2008, CureVac began collaborating with Lothar Stitz, director of the Institute of Immunology and professor of virology and immunology at the Loeffler Institute, to design and test mRNA-based vaccines to prevent influenza.

Hoerr said that the collaboration brought CureVac infectious disease expertise it did not have in-house. "Our strength is in the technology itself, in mRNA and chemical formulations, and also in understanding the immune response. But we needed expertise in animal challenge models, and, by collaborating with academics, we were able to gain access to that. In addition, it was important to show that another group could use our mRNA approach and get the same results—it is proof of concept that we can distribute the technology."

CureVac worked with Stitz's group to design and test mRNA vaccines encoding the influenza A virus hemagglutinin (HA), influenza A virus neuraminidase (NA) and influenza A virus nucleoprotein (NP) antigens. The vaccines incorporated the company's RNActive design technology, which includes optimizing mRNA base-pair content, engineering untranslated regions (UTRs) and complexing with protamine, an arginine-rich protein that binds and stabilizes mRNA.

In mice, vaccination with a single dose of mRNA encoding both HA and NA antigens led to complete protection against strain-matched

**EDITORIAL****Editor-in-Chief:** Karen Bernstein, Ph.D.**Managing Editor:** Gaspar Taroncher-Oldenburg, Ph.D.**Executive Editor:** Steve Edelson**Senior Editors:** Tracey Baas, Ph.D.; Joanne Kotz, Ph.D.**Writers:** Chris Cain, Ph.D.; Michael Flanagan; Tim Fulmer, Ph.D.;

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PO Box 1246
San Carlos, CA 94070-1246
T: +1 650 595 5333Chadds Ford
223 Wilmington-West Chester Pike
Chadds Ford, PA 19317
T: +1 610 558 1873Chicago
20 N. Wacker Drive, Suite 1465
Chicago, IL 60606-2902
T: +1 312 755 0798Oxford
287 Banbury Road
Oxford OX4 7JA
United Kingdom
T: +44 (0)18 6551 2184Washington, DC
2008 Q Street, NW, Suite 100
Washington, DC 20009
T: +1 202 462 9582**Nature Publishing Group**New York
75 Varick Street, 9th Floor
New York, NY 10013-1917
T: +1 212 726 9200London
The Macmillan Building
4 Crinan Street
London N1 9XW
United Kingdom
T: +44 (0)20 7833 4000Tokyo
Chiyoda Building 6F
2-37 Ichigayatamachi
Shinjuku-ku, Tokyo 162-0843
Japan
T: +81 3 3267 8751

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influenza infection, whereas mice injected with control antigen died.

In ferrets, which more closely model human infection, injection with an mRNA-encoded HA vaccine induced an antibody titer that would meet requirements for licensure under EMA guidelines. In pigs, injection of a vaccine containing HA, NA and NP prevented disease, whereas unvaccinated pigs showed mild to moderate signs of infection.

Josef Thalhamer, professor of allergy and immunology at the **University of Salzburg**, told *SciBX* that the data make a compelling case for further exploration of the mRNA vaccine. "The data are strong and clearly indicate the induction of protective immunity after vaccination with an mRNA vaccine. In particular, the results of the immunizations in pigs can be considered a breakthrough. They demonstrate that a moderate dose of an mRNA vaccine can trigger protective antibody responses against infectious diseases in large animals, which suggests this vaccine type may be effective in humans as well."

Results were published in *Nature Biotechnology*.

Broad application

The mRNA vaccine could offer a host of potential manufacturing and efficacy advantages over conventional flu vaccines.

The researchers demonstrated that the mRNA vaccine could be stored for 3 weeks at 37 °C without losing efficacy. Stitz added that production of clinical-grade vaccine could be achieved within 6–8 weeks of the identification of influenza antigen sequence.

Methods for rapid influenza vaccine production are sorely needed. In 2009, the **World Health Organization** said it would take about [five to six months for the first supplies of approved vaccine to become available](#) once a new strain of influenza virus with pandemic potential is identified and isolated.

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The new product also could offer longer-term, broader protection against influenza than traditional vaccines.

“The problem with the conventional vaccines is that the virus needs to be inactivated, and dead vaccines only induce a strong neutralizing B cell response but no T cell response,” noted Stitz. “The nice thing about this approach is that mRNA actually mimics an infection, as the respective protein coded for in the mRNA is synthesized within the cell, so the antigen can both induce antibodies and enter the MHC antigen presentation pathway for T cells.”

Stitz’s team demonstrated this response by injecting mice with an mRNA vaccine encoding NP, which provided T cell–dependent protection from infection with a heterologous influenza strain.

Christian Mandl, VP and global head of virology at **Novartis AG**’s Vaccines & Diagnostics Inc. division, agreed that a broad, prolonged immune response is a key potential advantage for RNA vaccines.

“Expression of antigen *in situ* by nucleic acid vaccines, including RNA, has the potential to provide prolonged antigenic stimulus in contrast to protein-based vaccines, which are injected as a bolus and typically cleared rapidly,” he said. “The differential kinetics of antigen exposure between RNA- and protein-based vaccines could lead to more potent and durable immune responses.”

He added, “Preclinical work on RNA prophylactic and therapeutic vaccines has demonstrated their ability to elicit potent and broad immune responses, including functional antibodies, Th1-type T cell responses and cytotoxic T cells. Hence, RNA vaccines have the potential as a platform technology to address a wide variety of infectious diseases caused by viruses, bacteria and parasites, as well as noninfectious diseases such as cancer.”

Novartis did not disclose the status of its RNA vaccine programs, but earlier this year Mandl’s team published an extensive review on the topic and also described the development of a self-replicating RNA vaccine that protected mice from respiratory syncytial virus (RSV) infection.^{5,6}

Gary Nabel, who stepped down this month as director of the Vaccine Research Center at the **NIH** to become SVP and CSO at Sanofi, told *SciBX* he was impressed with the progress on RNA vaccines. “It’s really pretty remarkable that the RNA technology is working. I think it’s a very promising development. We all think of RNA as being incredibly unstable, so it’s pretty mindboggling that you could do this with mRNA. The advances have come from stabilizing the RNA and engineering it to ensure it is efficiently translated.”

Nabel wants to see results in humans. “The contributions of T cells to flu vaccine efficacy are more theoretical at this point than proven. There are reasons to think it’s a good thing, but most animal studies show that a good antibody response is the main component you need for protection. The bottom line is that the jury is still out on what it really can do, and until you test it in people, you don’t know what it can do.”

Next steps

CureVac is now collaborating with Sanofi’s Pasteur vaccines division, **In-Cell-Art S.A.S.** and the **U.S. Department of Defense**’s Defense Advanced Research Projects Agency (DARPA) to develop mRNA vaccines against undisclosed pathogens through the RN Armor Vax consortium.

The consortium was established last November with a total budget of \$33.1 million. For new technology developed under the project, DARPA will receive a nonexclusive license for U.S. government use, which excludes commercial purposes. Sanofi has commercial options to use CureVac’s RNActive technology to develop vaccines for predefined pathogens.

CureVac is eligible for up to €150.5 million (\$206.6 million) per pathogen in upfront and milestone payments from Sanofi, plus tiered royalties. In-Cell-Art, a French company that specializes in macromolecular delivery technology, will provide Sanofi with undisclosed formulation technologies for mRNA-based vaccines and is eligible for undisclosed upfront payments, milestones and royalties.

Hoerr did not disclose whether influenza was among the predefined pathogens included in the consortium but said it was a logical pathogen to select for proof-of-concept studies because of the wealth of existing preclinical data available for comparison.

He told *SciBX* that CureVac is still focused on its clinical-stage oncology programs but sees the development of vaccines for infectious disease as a logical additional avenue for the company to pursue. The company’s lead prostate cancer program, CV9103, is a four-antigen, mRNA-encoded vaccine that is in Phase IIb to treat metastatic, castration-resistant prostate cancer (mCRPC). Results are expected in 2016.

Stitz said he plans to continue to collaborate with the company on potential mRNA vaccine candidates, and the group has unpublished data for the approach against rabies virus. He said his group also plans to explore the basic biology questions underlying the approach. These include further characterizing mRNA uptake by cells and determining where and through which signaling pathways the vaccine is acting to stimulate an immune response.

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REFERENCES

1. Petsch, B. *et al. Nat. Biotechnol.*; published online Nov. 25, 2012; doi:10.1038/nbt.2436
Contact: Lothar Stitz, Friedrich Loeffler Institute, Greifswald-Insel Riems, Germany
e-mail: lothar.stitz@fli.bund.de
Contact: Karl-Josef Kallen, CureVac GmbH, Tuebingen, Germany
e-mail: josef.kallen@curevac.com
2. Usdin, S. & McCallister, E. *BioCentury* 17(20), A1; May 4, 2009
3. Ulmer, J.B. *et al. Nat. Biotechnol.* 24, 1377–1383 (2006)
4. Kutzler, M.A. & Weiner, D.B. *Nat. Rev. Genet.* 9, 776–788 (2008)
5. Geall, A.J. *et al. Proc. Natl. Acad. Sci. USA* 109, 14604–14609 (2012)
6. Ulmer, J.B. *et al. Vaccine* 30, 4414–4418 (2012)

COMPANIES AND INSTITUTIONS MENTIONED

CureVac GmbH, Tuebingen, Germany
Friedrich Loeffler Institute, Greifswald-Insel Riems, Germany
In-Cell-Art S.A.S., Nantes, France
National Institutes of Health, Bethesda, Md.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
University of Salzburg, Salzburg, Austria
U.S. Department of Defense, Washington, D.C.
World Health Organization, Geneva, Switzerland

MRC Technology extends reach

By Tracey Baas, Senior Editor

After six years of focusing on developing small molecule drug candidates and humanizing therapeutic mAbs, both mostly from U.K. sources, MRC Technology, the commercialization arm of the country's Medical Research Council, is taking its search for new assets to Asia. MRC Technology has taken its first steps on that front through a deal with the **Shanghai Institute of Biochemistry and Cell Biology**, part of the **Chinese Academy of Sciences**.

The MRC is the U.K.'s largest publicly funded biomedical research organization. MRC Technology initially provided technology transfer services exclusively to MRC but now works with multiple charitable and academic organizations.

MRC Technology is a not-for-profit technology transfer company that offers IP management and commercial development of healthcare-related science to scientific organizations. The income generated helps fund further research in the originating scientific organizations.

The company has an in-house screening facility, chemical libraries of about 120,000 compounds and the medicinal chemistry expertise of researchers at MRC Technology's Centre for Therapeutics Discovery. In addition, MRC Technology has access to about 100,000 proprietary compounds that are target-based and 22 shelved compounds through a deal with **AstraZeneca plc**.

Table 1. MRC technology deals.

Year	Indication	Collaborator	Deal	Therapeutic modality
2012	Undisclosed	Shanghai Institute of Biochemistry and Cell Biology (SIBCB) , part of the Chinese Academy of Sciences	SIBCB will share access to potential targets, which can be further developed at MRC Technology's Centre for Therapeutic Discovery	Small molecules
2012	Infectious disease, drug addiction and autoimmune disease	Mount Sinai School of Medicine	MRC Technology will humanize mouse antibodies created by Mount Sinai's Center for Therapeutic Antibody Discovery to control infection and treat disease	mAbs
2012	Undisclosed	Academic research institutes	MRC Technology will provide academic research institutes free access to a representative subset of 9,000 compounds in plate format from its library of 150,000 small molecules. MRC Technology will have a right of first refusal to partner on any resulting drug development programs and also will have joint ownership of any generated data	Small molecules
2012	Undisclosed	William Harvey Research Institute at Barts and The London School of Medicine and Dentistry	MRC Technology will screen and develop compounds to target G protein-coupled receptors	Small molecules
2010	Neurology	Roche's Genentech Inc. unit	MRC Technology granted Genentech exclusive rights to develop and commercialize a group of small molecule drug candidates for the potential treatment of neurological diseases. Medical Research Council (MRC) will receive an upfront payment and is eligible for milestones and royalties	Small molecules
2010	Cancer, cardiovascular disease, neurology and infection	AstraZeneca plc	MRC Technology will screen a compound library for five undisclosed targets from AstraZeneca in the areas of cancer, cardiovascular disease, neurology and infection and five additional targets from MRC for the same areas. The library includes 100,000 compounds from the pharma and 50,000 compounds from MRC. The partners will retain ownership of their respective compounds and plan to negotiate licenses for projects chosen for further development. The licenses would trigger option fees to MRC	Small molecules
2008	Lymphoma	Biolex Therapeutics Inc.	MRC Technology will humanize Biolex's BLX-301 antibody, which is an optimized glycosylated version of rituximab and is in preclinical development for non-Hodgkin's lymphoma. Biolex will retain full rights to the antibody, and MRC will be eligible for milestones and royalties	mAbs
2007	Alzheimer's disease (AD)	Intellect Neurosciences Inc.	MRC Technology has humanized two of Intellect's mAbs against β -amyloid ($A\beta$) to treat AD and age-related macular degeneration (AMD). Intellect is working with Lonza Group Ltd. to manufacture humanized antibody and a humanized antibody conjugate, called conjumab A, for lead testing and optimization. MRC Technology is eligible for undisclosed milestones and royalties	mAbs
2007	Cancer	Akzo Nobel N.V.	MRC Technology will humanize an antibody from Akzo's Organon BioSciences N.V. unit to treat cancer. MRC Technologies is eligible for milestones and royalties. Organon has all rights to develop and commercialize the humanized antibody. Organon was acquired by Schering-Plough Corp. (now part of Merck & Co. Inc.)	mAbs

Under MRC Technology's deal with the Shanghai Institute of Biochemistry and Cell Biology (SIBCB) to identify new targets for drug development, the institute will share access to potential new targets, which can be further developed at the Centre for Therapeutics Discovery.

"We are always looking for innovative science that might be attractive to pharmaceutical companies further down the drug discovery pipeline," said Michael Dalrymple, director of business development at MRC Technology.

According to Zi Zhang, MRC Technology's business development manager in charge of the partnership, "We chose SIBCB because of its long history and past achievements and because of it being recognized as a flagship institute by the Chinese life science community."

MRC Technology said it has not yet discussed financial terms or rights to IP with SIBCB.

"MRC Technology and SIBCB will share access to those potential targets and secondary assays. Any tool compounds found can be used to validate those targets in collaboration with the original principal investigator," noted Dalrymple. "Ultimately, MRC Technology's collaborative drug discovery engine relies on identifying top scientists and institutes so that we can plug into the cutting-edge biology and, working with the scientists, identify good targets."

He added, "At this stage we are target and indication agnostic. We're really just looking for interesting science that could provide potential targets for unmet medical needs. While we're not focused on unmet medical needs of China per se, if those targets shake out first, even better."

"We hope to show that collaborations in China can be successful by identifying innovative druggable targets with SIBCB and developing

potent and selective novel therapeutics as quickly as we can—18–36 months would be ideal," said Dalrymple. "If or when we do show our Shanghai collaboration is successful, we already have a short list of other Chinese institutes we'd also like to reach out to."

U.S. deal

Earlier this year, MRC Technology announced a partnership to apply MRC Technology's antibody humanization capabilities to **Mount Sinai School of Medicine's** mouse antibodies against targets in infectious disease, drug addiction and autoimmune disease (see Table 1, "MRC Technology deals").

Mount Sinai will generate mAbs against targets that have successfully been through MRC Technology's antibody review process and selected for development. MRC Technology will provide humanization by altering the mouse antibody's molecular structure to make it compatible for therapeutic use in humans without affecting its binding specificity.

A number of MRC Technology's collaborations already have progressed beyond the screening phase at the Centre for Therapeutics Discovery. Ongoing programs include 10 different targets and span 7 indications (see Table 2, "Centre for Therapeutics Discovery").

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COMPANIES AND INSTITUTIONS MENTIONED

AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.

Chinese Academy of Sciences, Beijing, China

Medical Research Council, London, U.K.

Mount Sinai School of Medicine, New York, N.Y.

MRC Technology, London, U.K.

Shanghai Institute of Biochemistry and Cell Biology, Shanghai, China

Table 2. Centre for Therapeutics Discovery.

Target	Project	Stage of development	Collaborator
Undisclosed	Undisclosed	Hit to lead	Undisclosed
Melanocortin 3 receptor (MC3R)	MC3R for inflammatory and mechanical joint disease	Hit to lead	William Harvey Research Institute at Barts and The London School of Medicine and Dentistry
Formyl peptide receptor-like 1 (FPRL1; FPR2)	FPR2 agonists for ischemia/reperfusion injury	Hit to lead	William Harvey Research Institute
<i>Mycobacterium tuberculosis</i> glucanase GlgE (glgE)	Inhibitors of glgE to treat tuberculosis	Hit to lead	John Innes Centre
Melanocortin 2 receptor (MC2R)	MC2R for Cushing's syndrome	Hit to lead	Queen Mary, University of London
MAP/microtubule affinity-regulating kinase (MARK)	Inhibitors of MARK microtubule-associated protein- τ (MAPT; TAU; FTDP-17) phosphorylation in Alzheimer's disease (AD)	Hit to lead	MRC Protein Phosphorylation Unit
Neurotrophic tyrosine kinase receptor 1 (NTRK1; TrkA)	TrkA modulators for pain	Hit to lead	University of Bristol
Galanin receptor 1 (GALR1)	New allosteric modulators of the second galanin receptor subtype (GALR2) to treat pain	Screening	University of Bristol
Potassium channel Kir7.1 (KCNJ13)	Inwardly rectifying KCNJ13 to treat postpartum hemorrhage	Screening	The University of Warwick
<i>Plasmodium falciparum</i> exported protein 2 (PKG 123420; EXP2)	Inhibitors of PKG to treat malaria	Hit to lead	London School of Hygiene & Tropical Medicine

Losing sleep over flumazenil

By Lauren Martz, Staff Writer

Emory University School of Medicine researchers have identified excessive daytime sleepiness as a repurposing opportunity for flumazenil, a generic GABA_A receptor antagonist.¹ Although safety issues have stymied prior attempts to use GABA_A receptor antagonists in this chronic indication, the group thinks a new formulation could make it possible.

Flumazenil is the only FDA-approved GABA_A antagonist. It is a competitive antagonist that targets the benzodiazepine binding site on GABA_A receptors and is marketed to treat sedative overdose and improve recovery from anesthesia.

Previous studies have shown that flumazenil increases vigilance in patients with sleep deprivation, idiopathic recurrent stupor and hepatic encephalopathy.²⁻⁴ However, the drug's side effects include anxiety and seizures. Those adverse events plus the short-lived activity of the drug have discouraged its development for sleep disorders.

Nevertheless, David Rye and colleagues at Emory set out to determine whether flumazenil could reverse the excessive GABA_A receptor activity that might underlie conditions including idiopathic hypersomnia and narcolepsy without cataplexy. Both are types of excessive daytime sleepiness for which existing therapeutics are ineffective.

Rye is a professor of neurology at Emory University School of Medicine and director of research at Emory Healthcare's Program in Sleep Medicine.

To confirm that GABA_A activity was elevated in the CNS of patients with hypersomnia, Rye's team cultured human embryonic kidney cells expressing γ -aminobutyric acid (GABA) family receptors in the presence of cerebrospinal fluid (CSF) from hypersomnolent patients plus 10 μ M exogenous GABA_A.

The combination of GABA_A plus CSF from patients increased GABA_A signaling more than GABA_A alone or GABA_A plus CSF from healthy controls. In the same cells, flumazenil reversed the effects of patient CSF on GABA_A signaling.

Next, Rye and colleagues treated seven hypersomnia patients with a single injection of saline plus flumazenil. The patients showed improved vigilance and subjective alertness following treatment.

Finally, the team looked at whether flumazenil had long-term efficacy. Due to the short half-life of injectable flumazenil, they generated sublingual and transdermal formulations of the drug. In a single patient with idiopathic hypersomnia, the new formulations given every two to three hours while the patient was awake reduced excessive daytime sleepiness over four years.

Rye told *SciBX* that "the next steps include conducting a larger, double-blind, placebo-controlled trial." Details of the trial are not yet decided, he said, but the study likely would be run by Emory University or as part of the National Institute of Neurological Disorders and Stroke's Network for Excellence in Neuroscience Clinical Trials (NeuroNEXT) consortium. The trial is planned for the fall of 2013 at the earliest.

Results were published in *Science Translational Medicine*.

Path to repurposing

Although the findings point to the potential to repurpose flumazenil, the clinically approved version of the compound is intravenous, short lived and intended for single-injection applications. Moreover, the drug carries a black box warning of seizure risk.

"Flumazenil has limited value to these patients because it has a short half-life and rapid metabolism," said Bjarke Ebert, senior medical advisor at H. Lundbeck A/S.

Thus, he said, the Emory researchers "need to confirm that their sublingual version and transdermal formulation lead to a steady-state blockade of the benzodiazepine site."

Lundbeck markets Tranxene clorazepate dipotassium, an oral benzodiazepine, to treat anxiety disorders.

"It might be worth seeking compounds with the same mechanism as flumazenil that are longer acting," suggested Karl-Uwe Petersen, VP of pre-clinical development at Paion AG's Paion Deutschland GmbH subsidiary.

Rye said his team is considering screening for new compounds with the same mechanism of action. "Ideally, discovering more details about the pharmacological targets could allow probing from additional molecular libraries for alternative promising treatments," he said.

Paion and Ono Pharmaceutical Co. Ltd. have completed Phase II testing of Remimazolam, a short-acting general anesthetic and sedative that activates the GABA_A receptor.

On the safety front, seizure risk could be addressed with a dose-escalation trial of flumazenil with continuous electroencephalography or brain imaging surrogate endpoints, said Matt Bianchi, assistant professor of neurology in the Sleep Division at Massachusetts General Hospital.

Despite the safety hurdles, flumazenil could have an advantage over other hypersomnia therapeutics because it targets the pathological mechanisms that underlie the condition.

"The issue with current stimulatory agents is that they are not useful in all subjects, and they do not directly address the problem that has been identified in the types of hypersomnia patients described in the paper," said Petersen.

He added, "The difference between GABA_A modulators and existing strategies to treat daytime sleepiness is that most therapies are aimed at increasing excitation, while GABA_A modulators are aimed at reducing the dampening effect on excitation. The problem with excitatory stimulation is that it is not a very direct method. It really just increases consciousness and requires high doses that could lead to side effects. For example, the widely used modafinil can cause headaches, changes in blood pressure and overstimulation that can cause nervousness and sleeplessness."

Teva Pharmaceutical Industries Ltd. markets Provigil modafinil to treat narcolepsy, shift work sleep disorder and excessive sleepiness associated with obstructive sleep apnea.

Ebert added, "It is clear that modafinil works for a lot of patients with excessive daytime sleepiness, but it is not exactly clear how. For flumazenil, we have the mechanism of action, and it appears that the drug might actually address the pathophysiology of the disease."

"Ideally, discovering more details about the pharmacological targets could allow probing from additional molecular libraries for alternative promising treatments."

—David Rye,
Emory University
School of Medicine

Rye said that although it is hard to compare the efficacy of drugs across different types of hypersomnias, his team found that flumazenil improved vigilance in patients with sleepiness attributed to shift work. In 2005, a group from **Harvard Medical School** and **Cephalon Inc.** found a smaller impact of modafinil on vigilance in patients with the same condition.⁵

Emory University has patented the work, and the IP is unlicensed.

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REFERENCES

1. Rye, D.B. *et al. Sci. Transl. Med.*; published online Nov. 21, 2012; doi:10.1126/scitranslmed.3004685
Contact: David B. Rye, Emory University School of Medicine, Atlanta, Ga.
e-mail: drye@emory.edu
2. Ahboucha, S. & Butterworth, R.F. *Metab. Brain Dis.* **20**, 425–437 (2005)

3. Seifritz, E. *et al. Psychopharmacology (Berl.)* **120**, 449–456 (1995)
4. Cortelli, P. *et al. Sleep Med. Rev.* **9**, 477–487 (2005)
5. Czeisler, C.A. *et al. N. Engl. J. Med.* **353**, 476–486 (2005)

COMPANIES AND INSTITUTIONS MENTIONED

Cephalon Inc. (NASDAQ:CEPH), Frazer, Pa.
Emory Healthcare, Atlanta, Ga.
Emory University, Atlanta, Ga.
Emory University School of Medicine, Atlanta, Ga.
Harvard Medical School, Boston, Mass.
H. Lundbeck A/S (CSE:LUN), Copenhagen, Denmark
Massachusetts General Hospital, Boston, Mass.
National Institute of Neurological Disorders and Stroke, Bethesda, Md.
Ono Pharmaceutical Co. Ltd. (Tokyo:4528; Osaka:4528), Osaka, Japan
Paion AG (Xetra:PA8), Aachen, Germany
Teva Pharmaceutical Industries Ltd. (NYSE:TEVA), Petah Tikva, Israel

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Targeting interleukins in Alzheimer's disease

By Kai-Jye Lou, Staff Writer

Researchers at **Charité–University Hospital Berlin** and the **University of Zurich** have data showing that p40, a protein subunit shared by IL-12 and IL-23, could be a new therapeutic target in Alzheimer's disease.¹ The group now is trying to better understand the downstream mediators of the pathway and wants to find an industry partner to test late-stage or marketed p40-targeting therapies, such as **Johnson & Johnson's** psoriasis drug Stelara ustekinumab.

p40 is produced by microglia and is a protein subunit shared by IL-12 and IL-23. Microglia are the resident macrophages of the CNS, and aberrant phenotypes are linked to multiple neurodegenerative diseases, including Alzheimer's disease (AD).² These CNS cells accumulate around β -amyloid ($A\beta$) plaques and are known to be a major source of proinflammatory cytokines,³ although their precise role in AD pathogenesis and progression is unclear.⁴

$A\beta$ plaques are hallmarks of AD and trigger inflammatory responses.⁵

A research group co-led by Frank Heppner has been trying to characterize the role of microglia in $A\beta$ pathology in AD. Initially, it seemed there was at least no immediate role. In 2009, his group reported that ablation of the microglia cell population for four weeks had no observable effect on $A\beta$ plaque load and amyloid-associated neurotoxicity in two mouse models of AD.⁶

Now, however, his team has shown that targeting a cytokine subunit produced by aberrant microglia for at least 60 days reduces $A\beta$ plaque loads and reverses AD pathology.

In a transgenic mouse model of AD, microglial production of p40—as well as levels of IL-12 and IL-23—was greater than that in wild-type controls.

“We found that these molecules were upregulated in a distinct subpopulation of microglia in this mouse model of Alzheimer's and thus thought they may be associated with disease pathology,” said Heppner, chair of the Department of Neuropathology at Charité–University Hospital Berlin. “Thus, we sought to also investigate what happens when we inhibit the signaling of these molecules in the mouse model—first genetically and then pharmacologically after seeing positive effects.”

Peripheral delivery of an antibody against p40 decreased $A\beta$ plaque burden in young mice compared with delivery of an isotype control. In the same model using aged mice, intracerebroventricular delivery of the p40-targeting antibody decreased both cognitive deficits and soluble $A\beta$ burden compared with what was seen in controls.

Behavioral assessments were not carried out in the young mice as the AD-associated cognitive deficits in this model are not detectable until

the animals are older. The group did not evaluate peripheral delivery of the p40-targeting antibody in the aged mice.

The link between p40 and AD also carried over to patients.

An analysis of cerebrospinal fluid (CSF) samples from 39 patients with AD and 20 non-AD controls showed an association between elevated levels of p40 and reduced cognitive performance ($p < 0.05$).

Results were published in *Nature Medicine*.

“Our study not only helps to confirm that inflammation is a crucial mediator of Alzheimer's pathophysiology but also shows what type of molecule to target in the immune system in this disease setting,” said Heppner, a co-corresponding author. “Our results clearly show for the first time a strong association of IL-12 and IL-23 with Alzheimer's and identify p40 as a candidate therapeutic target for the disease.”

“The data in their mouse model studies are very strong and show that targeted regulation of microglial function leads to improvements in Alzheimer's pathophysiology,” added Terrence Town, a professor of biomedical sciences and chair in regenerative medicine at the Regenerative Medicine Institute at **Cedars-Sinai Medical Center** and the **University of California, Los Angeles David Geffen School of Medicine**.

“They also looked at CSF samples from humans, lending support to the case that what they see in mice is also happening in humans.”

“We look forward to seeing these preclinical results reproduced and confirmed with the hope that they will shed more light on the mechanistic role of inflammation in cognitive disorders and that they will help provide an informed foundation for successful translation to clinical therapeutic application for drugs like Stelara,”

said J&J spokesperson Brian Kenney.

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— Terrence Town,
Cedars-Sinai Medical Center

p40 questions

Stelara is marketed to treat plaque psoriasis and is under review to treat active psoriatic arthritis. The mAb also is in Phase III testing for Crohn's disease.

The other late-stage, p40-targeting mAb is **Abbott Laboratories'** Ozespa briakinumab, which is in Phase II testing for Crohn's disease. Abbott withdrew a BLA in the U.S. and an MAA in the EU for Ozespa to treat psoriasis in January 2011 after the agencies asked for additional data and analyses.

“This isn't just another study where you look at the results and in 15 years, maybe you will have something in the clinic,” said Richard Ransohoff, director of the Neuroinflammation Research Center at the **Cleveland Clinic**. “Instead, this is a study where reagents against the newly identified target are already available. With careful thought and trial design, they could begin testing their therapeutic hypothesis in a translational program.”

An open question is how to best deliver a mAb-based therapy across the blood brain barrier (BBB).

“When we applied our antibody peripherally in the mouse model, we found that it had a significant effect in reducing plaque burden in the brain, which suggests that sufficient amounts of the antibody could be getting across the blood brain barrier and that peripheral application might be enough,” Heppner told *SciBX*.

Town said that intrathecal delivery is another delivery option and added that other research groups already are working on strategies to deliver mAbs and other large-molecule therapeutics across the BBB and into the CNS.

One such approach is the molecular Trojan horse drug delivery technology being developed by **ArmaGen Technologies Inc.**^{7,8} The technology involves creating a fusion protein of a therapeutic compound and a Trojan horse molecule that targets surface receptors on endothelial cells to facilitate transport across the BBB.

Another strategy, being developed by researchers at **Cornell University**, involves temporarily agonizing adenosine receptors in the brain's vasculature to loosen the BBB.^{9,10}

Alternatively, small molecule inhibitors of IL-12 and IL-23 could be easier to get across the BBB than antibodies.

At least one company—**Synta Pharmaceuticals Corp.**—has small molecule inhibitors of IL-12 and IL-23 in preclinical development to treat various autoimmune and inflammatory diseases. The company also previously developed apilimod, a small molecule that inhibits IL-12 and IL-23 production, to treat plaque psoriasis, Crohn's disease, common variable immunodeficiency (CVID) and rheumatoid arthritis (RA). The compound failed in Phase II trials in all four indications.

Michael Heneka, a professor of clinical neurosciences at the **University of Bonn**, thinks it is still too early to say whether targeting p40 will pan out in the AD setting and said the real question is when—and not how—to inhibit p40 in AD.

“Further studies will have to identify the precise time course of this particular immunological pathway in AD,” he told *SciBX*. “I would want to see studies in human samples to determine at what stages of AD and pre-AD this cytokine signaling pathway is active and how signaling in the pathway changes at different stages of the disease. I would also like to see an assessment of how this pathway affects different brain regions and a study to determine this pathway's relevance to sporadic and familial cases of AD.”

Ransohoff said it will be important to determine the cells that respond to IL-12 and IL-23 signaling in the AD setting and to further elucidate how signaling through these cytokines contributes to disease pathology.

Town said additional validation studies in postmortem brain tissue from patients with AD could help determine the cells responsible for producing IL-12 and IL-23 in close vicinity to the plaque lesions.

Heppner's group now is trying to replicate the study results using a

different mouse AD model with later disease onset. His group also is continuing with basic science studies to better characterize the function of p40, IL-23 and IL-23 downstream of microglia.

“What we want to do is to study downstream effects and mechanisms in this pathway,” he told *SciBX*. “The other axis we are interested in is finding a partner from industry to help set up a clinical trial, for example, to evaluate an existing p40-targeting antibody or similar compounds in Alzheimer's patients.”

The University of Zurich and Charité–University Hospital Berlin have cofiled a patent covering modulators of IL-12 and/or IL-23 to treat and prevent AD. The work is available for licensing from **Unitectra**, the technology transfer office of the University of Zurich.

Lou, K.-J. *SciBX* 5(49); doi:10.1038/scibx.2012.1276

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REFERENCES

1. Von Berg, J. *et al. Nat. Med.*; published online Nov. 25, 2012; doi:10.1038/nm.2965
Contact: Frank L. Heppner, Charité–University Hospital Berlin, Berlin, Germany
e-mail: frank.heppner@charite.de
Contact: Burkhard Becher, University of Zurich, Zurich, Switzerland
e-mail: becher@immunology.uzh.ch
2. Perry, V.H. *et al. Nat. Rev. Neurol.* **6**, 193–201 (2010)
3. Streit, W.J. *et al. J. Neuroinflammation* **1**, 14 (2004)
4. Jucker, M. & Heppner, F.L. *Neuron* **59**, 8–10 (2008)
5. Hickman, S.E. *et al. J. Neurosci.* **28**, 8354–8360 (2008)
6. Grathwohl, S.A. *et al. Nat. Neurosci.* **12**, 1361–1363 (2009)
7. Boado, R.J. *Drug News Perspect.* **21**, 489–503 (2008)
8. Fulmer, T. *SciBX* **1**(41); doi:10.1038/scibx.2008.991
9. Carman, A.J. *et al. J. Neurosci.* **31**, 13272–13280 (2011)
10. Osherovich, L. *SciBX* **4**(39); doi:10.1038/scibx.2011.1080

COMPANIES AND INSTITUTIONS MENTIONED

Abbott Laboratories (NYSE:ABT), Abbott Park, Ill.
ArmaGen Technologies Inc., Santa Monica, Calif.
Cedars-Sinai Medical Center, Los Angeles, Calif.
Charité–University Hospital Berlin, Berlin, Germany
Cleveland Clinic, Cleveland, Ohio
Cornell University, Ithaca, N.Y.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
Synta Pharmaceuticals Corp. (NASDAQ:SNTA), Lexington, Mass.
Unitectra, Zurich, Switzerland
University of Bonn, Bonn, Germany
University of California, Los Angeles David Geffen School of Medicine, Los Angeles, Calif.
University of Zurich, Zurich, Switzerland

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
Autoimmune disease				
Multiple sclerosis (MS)	4-1BB ligand (4-1BBL; TNFSF9; CD137L)	<p>Mouse studies suggest inhibiting CD137L signaling could help treat MS. In a mouse model of experimental autoimmune encephalomyelitis (EAE), <i>Cd137l</i> knockout decreased leukocyte cytokine secretion, infiltration in the spinal cord and disease severity compared with no knockout. Next steps include evaluating whether an anti-CD137L mAb or a soluble tumor necrosis factor receptor superfamily member 9 (TNFRSF9; 4-1BB; CD137) could help treat EAE.</p> <p>SciBX 5(49); doi:10.1038/scibx.2012.1277 Published online Dec. 20, 2012</p>	Unpatented; available for licensing	<p>Martínez Gómez, J.M. <i>et al. J. Neurosci.</i>; published online Dec. 12, 2012; doi:10.1523/JNEUROSCI.2473-12.2012 Contact: Herbert Schwarz, National University of Singapore, Singapore e-mail: phssh@nus.edu.sg</p>
Cancer				
Acute myelogenous leukemia (AML)	Unknown	<p>Cell culture and mouse studies suggest the generic malaria drug mefloquine could help treat AML. In cultured AML cell lines, mefloquine inhibited cell growth at low micromolar concentrations and disrupted lysosome integrity. In a mouse model of AML, mefloquine decreased tumor volume compared with vehicle control. Next steps include identifying and testing mefloquine analogs that more potently disrupt lysosomes.</p> <p>SciBX 5(49); doi:10.1038/scibx.2012.1278 Published online Dec. 20, 2012</p>	Unpatented; licensing status not applicable	<p>Sukhai, M.A. <i>et al. J. Clin. Invest.</i>; published online Dec. 3, 2012; doi:10.1172/JCI64180 Contact: Aaron D. Schimmer, University Health Network, Toronto, Ontario, Canada e-mail: aaron.schimmer@utoronto.ca</p>
Renal cell carcinoma (RCC)	Sialidase 3 (NEU3; SIAL3)	<p>Studies in cell culture suggest antagonizing NEU3 could help treat RCC. In cultured RCC cells, small hairpin RNA knockdown of NEU3 decreased signaling through oncogenic pathways, expression of tumor-associated markers and tumor invasiveness compared with no knockdown. Next steps could include identifying NEU3 inhibitors and testing them in cultured RCC cells.</p> <p>SciBX 5(49); doi:10.1038/scibx.2012.1279 Published online Dec. 20, 2012</p>	Patent and licensing status unavailable	<p>Tringali, C. <i>et al. J. Biol. Chem.</i>; published online Nov. 8, 2012; doi:10.1074/jbc.M112.407718 Contact: Bruno Venerando, University of Milan, Milan, Italy e-mail: bruno.venerando@unimi.it</p>
Sarcoma	Insulin-like growth factor 1 receptor (IGF1R; CD221); arrestin β 1 (ARRB1); MAPK (ERK)	<p>Cell culture studies suggest inhibiting ARRB1 or ERK could help increase the efficacy of IGF1R-targeting molecules. Therapeutic antibodies that exert their effect through IGF1R also can downregulate expression of the receptor, which could impede therapeutic efficacy over time. In Ewing's sarcoma cell lines treated with Pfizer Inc.'s IGF1R antibody figitumumab, IGF1R downregulation was mediated by ERK activation caused by ARRB1 recruitment. In the same cell lines, figitumumab and an ERK inhibitor decreased cell viability compared with figitumumab alone. Next steps include extending the studies to animal models of other cancers.</p> <p>Pfizer discontinued a Phase III trial of figitumumab in non-small cell lung cancer (NSCLC) in 2010.</p> <p>At least 14 companies have IGF1R antagonists or antibodies in development stages ranging from preclinical to marketed to treat various indications.</p> <p>SciBX 5(49); doi:10.1038/scibx.2012.1280 Published online Dec. 20, 2012</p>	Patent and licensing status undisclosed	<p>Zheng, H. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Nov. 27, 2012; doi:10.1073/pnas.1216348110 Contact: Leonard Girnita, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden e-mail: leonard.girnita@ki.se</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cardiovascular disease				
Myocardial infarction (MI); ischemia/reperfusion injury	MicroRNA-199a-3p (miR-199a-3p); miR-590-3p	Rodent studies suggest exogenous human miR-590-3p and miR-199a-3p could help promote cardiac repair following MI. In rat cardiomyocytes, vector-induced expression of human miR-590-3p or miR-199a-3p both increased proliferation compared with expression of control miRNA. In a mouse model of MI, viral vector-induced expression of miR-590-3p or miR-199a-3p decreased infarct size and increased cardiac function compared with expression of control miRNA. Next steps include developing a pharmaceutical formulation of the miRNAs, assessing the best route of delivery and evaluating efficacy in larger animal models. SciBX 5(49); doi:10.1038/scibx.2012.1281 Published online Dec. 20, 2012	Patent pending covering use of miRNAs for inducing cardiomyocyte proliferation for use in cardiovascular indications; available for licensing	Eulalio, A. <i>et al. Nature</i> ; published online Dec. 5, 2012; doi:10.1038/nature11739 Contact: Mauro Giacca, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy e-mail: giacca@icgeb.org
Endocrine/metabolic disease				
Diabetes	Fibroblast growth factor receptor 1 (FGFR1; CD331); klotho- β (KLB); fibroblast growth factor 21 (FGF21)	<i>In vitro</i> and nonhuman primate studies identified an agonistic mAb of the FGFR1-KLB complex that could help treat type 2 diabetes. <i>In vitro</i> screening of antibodies from humanized mice identified an antibody that specifically bound to and agonized the FGFR1-KLB complex, which is a target of FGF21 expressed in adipocytes. In obese monkeys, the antibody decreased body weight, BMI, insulin resistance and triglyceride levels compared with vehicle. Next steps at Amgen Inc. include longer-term preclinical studies. Ambrx Inc. and Bristol-Myers Squibb Co. have ARX618, a stabilized form of FGF21, in preclinical development for type 2 diabetes. Roche's Genentech Inc. has an agonistic antibody that targets FGFR1 in preclinical development for the same indication. SciBX 5(49); doi:10.1038/scibx.2012.1282 Published online Dec. 20, 2012	Patent application filed; licensing status undisclosed	Foltz, I.N. <i>et al. Sci. Transl. Med.</i> ; published online Nov. 28, 2012; doi:10.1126/scitranslmed.3004690 Contact: Yang Li, Amgen Inc., South San Francisco, Calif. e-mail: yangl@amgen.com
Diabetes; obesity	G protein-coupled receptor family C group 5 member B (GPRC5B)	Mouse studies suggest inhibiting GPRC5B could help treat obesity and type 2 diabetes. In mice fed a high-fat diet, <i>Gprc5b</i> knockout led to faster glucose removal, lower circulating levels of insulin and glucose and 18% lower body weight than wild-type <i>Gprc5b</i> expression. Next steps could include identifying a GPRC5B inhibitor. SciBX 5(49); doi:10.1038/scibx.2012.1283 Published online Dec. 20, 2012	Patent and licensing status unavailable	Kim, Y.-J. <i>et al. Sci. Signal.</i> ; published online Nov. 20, 2012; doi:10.1126/scisignal.2003149 Contact: Yoshio Hirabayashi, RIKEN Brain Science Institute, Saitama, Japan e-mail: hirabaya@riken.jp
Infectious disease				
HIV/AIDS	HIV gp120; CD4	<i>Ex vivo</i> tissue and nonhuman primate studies suggest topical formulations of miniCD4 could be used to prevent HIV infection. miniCD4 is a CD4 mimetic that is built into a stabilizing 27-amino-acid scaffold and interacts with the CD4-binding site of HIV gp120. In human cervical or colorectal human tissue explants, pretreatment with the miniCD4 topical microbicide prevented infection of the tissues. In a macaque model of SIV infection, vaginal pretreatment with the miniCD4 microbicide one hour before high-dose challenge prevented infection in five out of six animals. Next steps include toxicology studies of the miniCD4 microbicide. SciBX 5(49); doi:10.1038/scibx.2012.1284 Published online Dec. 20, 2012	MiniCD4 peptides patented; available for licensing	Dereuddre-Bosquet, N. <i>et al. PLoS Pathog.</i> ; published online Dec. 6, 2012; doi:10.1371/journal.ppat.1003071 Contact: Loïc Martin, Institute of Biology and Technology Saclay, Gif sur Yvette, France e-mail: loic.martin@cea.fr

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Influenza virus	Protease-activated receptor 1 (PAR1)	<p>Mouse studies suggest inhibiting PAR1 could help treat influenza virus infection. In mouse models of influenza A virus infection, genetic deletion or pharmacological inhibition of Par1 attenuated inflammation and virus replication in the lung and protected against weight loss and death. The PAR1 inhibitor vorapaxar protected the mice from low pathogenic H1N1 and H3N2 infection as well as high pathogenic H5N1 or pandemic H1N1 infection. Next steps could include testing clinical-stage PAR1 inhibitors.</p> <p>In 2011, Merck & Co. Inc. discontinued dosing of patients in two Phase III trials of vorapaxar to prevent cardiovascular events because intracranial hemorrhage occurred in a subset of patients. In 2012, the pharma announced plans to file regulatory applications for the compound in the same indication but in a restricted patient population.</p> <p>SciBX 5(49); doi:10.1038/scibx.2012.1285 Published online Dec. 20, 2012</p>	Patented by the French National Institute for Agricultural Research (INRA); available for licensing	<p>Khoufache, K. <i>et al. J. Clin. Invest.</i>; published online Dec. 3, 2012; doi:10.1172/JCI61667</p> <p>Contact: Béatrice Riteau, University of Lyon and the French National Institute for Agricultural Research (INRA), Lyon, France e-mail: beatrice.riteau@univ-lyon1.fr</p>
Malaria	Unknown	<p>Mouse studies identified a series of bile acid trioxanes that could help treat malaria. In a mouse model of malaria infection, bile acid–based trioxanes that had shorter side-chain lengths and increased polarity showed greater potency against the <i>Plasmodium yoelii</i> parasite than those that had longer side chains and decreased polarity. In the mouse model, the most potent trioxanes provided 100% protection against infection when given at 24 mg/kg a day for 4 days. Next steps could include testing the compounds in additional models of malaria infection.</p> <p>SciBX 5(49); doi:10.1038/scibx.2012.1286 Published online Dec. 20, 2012</p>	Patent and licensing status unavailable	<p>Singh, C. <i>et al. J. Med. Chem.</i>; published online Nov. 19, 2012; doi:10.1021/jm301323k</p> <p>Contact: Chandan Singh, Council of Scientific and Industrial Research, Central Drug Research Institute, Lucknow, India e-mail: chandancdri@yahoo.com</p>
Tuberculosis	G protein–coupled receptor 109A (GPR109A; HM74A)	<p>A study in macrophages and mice suggests inhibiting GPR109A could help treat tuberculosis. In <i>Mycobacterium tuberculosis</i>–infected macrophages, small interfering RNA knockdown of the host protein GPR109A decreased the accumulation of lipid bodies, which are a nutrient source for the bacteria, compared with no knockdown. In mice infected with <i>M. tuberculosis</i>, the GPR109A inhibitor mepenzolate bromide decreased pathogen loads in the lung, liver and spleen compared with vehicle. Next steps could include developing GPR109A inhibitors with better pharmacokinetics and testing the longer-term effects of inhibiting GPR109A in animal models of <i>M. tuberculosis</i> infection.</p> <p>SciBX 5(49); doi:10.1038/scibx.2012.1287 Published online Dec. 20, 2012</p>	Patent and licensing status unavailable	<p>Singh, V. <i>et al. Cell Host Microbe</i>; published online Nov. 15, 2012; doi:10.1016/j.chom.2012.09.012</p> <p>Contact: Kanury V.S. Rao, International Centre for Genetic Engineering and Biotechnology, New Delhi, India e-mail: kanury@icgeb.res.in</p>
Musculoskeletal disease				
Musculoskeletal disease	C-type natriuretic peptide (CNP; NPPC); fibroblast growth factor receptor 3 (FGFR3; CD333)	<p><i>Ex vivo</i> and mouse studies suggest a CNP analog that antagonizes FGFR3 could help treat achondroplasia, the most common form of dwarfism. In femurs from a mouse model of achondroplasia with a gain-of-function mutation in FGFR3, the CNP analog increased bone length compared with vehicle. In that mouse model, subcutaneous injection of the CNP analog beginning at seven days of age led to increased bone length compared with vehicle injection. In those mice, the CNP injections also corrected skull and growth plate defects. Next steps could include testing the approach in additional models of achondroplasia.</p> <p>SciBX 5(49); doi:10.1038/scibx.2012.1288 Published online Dec. 20, 2012</p>	Patent and licensing status unavailable	<p>Lorget, F. <i>et al. Am. J. Hum. Genet.</i>; published online Nov. 29, 2012; doi:10.1016/j.ajhg.2012.10.014</p> <p>Contact: Laurence Legeai-Mallet, Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France e-mail: laurence.legeai-mallet@inserm.fr</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Neurology				
Alzheimer's disease (AD)	Amyloid precursor protein (APP); β -amyloid ($A\beta$)	Cell culture and mouse studies suggest hydroxypropyl- β -cyclodextran could help treat AD. In cultured cells overexpressing mutant human APP, hydroxypropyl- β -cyclodextran decreased membrane cholesterol and $A\beta$ levels compared with vehicle. In a mouse model of AD, the compound decreased $A\beta$ plaque load and increased learning and memory compared with vehicle. Next steps include modifying the structure of hydroxypropyl- β -cyclodextran to enable tracking of the molecule in the brain and to increase its penetration across the blood brain barrier. SciBX 5(49); doi:10.1038/scibx.2012.1289 Published online Dec. 20, 2012	Patent application filed; available for licensing	Yao, J. <i>et al. J. Exp. Med.</i> ; published online Dec. 3, 2012; doi:10.1084/jem.20121239 Contact: M. Flint Beal, Weill Cornell Medical College, New York, N.Y. e-mail: fbeal@med.cornell.edu Contact: Jiaqi Yao, same affiliation as above e-mail: jiy2006@med.cornell.edu
Alzheimer's disease (AD)	Apolipoprotein E (APOE)	Mouse studies suggest mAbs against APOE could help treat AD. In a mouse model of AD, treatment early in life with APOE mAbs led to higher microglial activity and lower levels of β -amyloid ($A\beta$) aggregation in the brain than control mAb treatment. Next steps include testing the mAbs in mice bearing human versions of APOE, including the AD-associated APOE $\epsilon 4$ allele. SciBX 5(49); doi:10.1038/scibx.2012.1290 Published online Dec. 20, 2012	Patent pending; available for licensing	Kim, J. <i>et al. J. Exp. Med.</i> ; published online Nov. 5, 2012; doi:10.1084/jem.20121274 Contact: David M. Holtzman, Washington University in St. Louis School of Medicine, St. Louis, Mo. e-mail: holtzman@neuro.wustl.edu
Alzheimer's disease (AD)	Triggering receptor expressed on myeloid cells 2 (TREM2)	Genetic association studies identified a <i>TREM2</i> variant that could be associated with AD. Whole-genome sequencing of samples from 3,550 Icelandic patients with AD and 8,888 controls at least 85 years of age without AD identified rs75932628-T, a missense mutation in <i>TREM2</i> , that was significantly associated with increased AD risk ($p=3.4\times 10^{-10}$). The association was confirmed in cohorts from the U.S., Norway, the Netherlands and Germany. In Icelandic individuals without AD between 80 and 100 years of age, cognitive decline was worse in carriers of the rs75932628-T mutation than in noncarriers ($p=0.003$). In a second study, sequencing <i>TREM2</i> in 1,092 patients with AD and 1,107 controls identified additional <i>TREM2</i> variants in the patients with AD and an association between rs75932628 and increased risk of AD ($p<0.001$). The association was confirmed in an additional cohort and a meta-analysis of a genomewide association study in AD. Next steps could include confirming the findings in additional patient cohorts. SciBX 5(49); doi:10.1038/scibx.2012.1291 Published online Dec. 20, 2012	Patent and licensing status for first study unavailable Findings from second study unpatented; licensing status not applicable	Jonsson, T. <i>et al. N. Engl. J. Med.</i> ; published online Nov. 14, 2012; doi:10.1056/NEJMoa1211103 Contact: Kari Stefansson, deCode genetics ehf, Reykjavik, Iceland e-mail: kstefans@decode.is Guerreiro, R. <i>et al. N. Engl. J. Med.</i> ; published online Nov. 14, 2012; doi:10.1056/NEJMoa1211851 Contact: John Hardy, UCL Institute of Neurology, London, U.K. e-mail: j.hardy@ucl.ac.uk
Neurology	Guanine nucleotide binding protein α -activating activity polypeptide olfactory type (GNAL)	Human genetic studies suggest mutations in <i>GNAL</i> could cause primary torsion dystonia, a musculoskeletal disorder characterized by repetitive motions and abnormal postures. Exosome sequencing of two families with primary torsion dystonia identified eight different mutations in <i>GNAL</i> that were associated with the condition. Next steps could include screening additional patients for mutations in this gene and developing a model that recapitulates the primary torsion dystonia phenotype. SciBX 5(49); doi:10.1038/scibx.2012.1292 Published online Dec. 20, 2012	Patent application filed; licensing status undisclosed	Fuchs, T. <i>et al. Nat. Genet.</i> ; published online Dec. 9, 2012; doi:10.1038/ng.2496 Contact: Laurie Ozelius, Mount Sinai School of Medicine, New York, N.Y. e-mail: laurie.ozelius@mssm.edu

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			
Identifying natural-product antibiotics through induced expression of biosynthetic gene clusters	Induced expression of biosynthetic gene clusters isolated from environmental DNA could help generate new antibiotic leads. Many biosynthetic gene clusters that have been identified by sequencing environmental DNA samples are not well expressed in <i>Escherichia coli</i> systems. To improve expression, transcriptional regulators within sequenced biosynthetic gene clusters were identified, cloned into a plasmid and then coexpressed in <i>Streptomyces albus</i> along with the corresponding gene cluster they were predicted to control. Screening and fractionation of these <i>S. albus</i> cultures identified a tetracyclic antibiotic, tetarimycin A, with an MIC of 0.78 µg/ml against methicillin-resistant <i>Staphylococcus aureus</i> (MRSA). Next steps include scaling this approach to look at a larger number of environmental DNA-derived gene clusters arising from a more diverse set of environments. SciBX 5(49); doi:10.1038/scibx.2012.1293 Published online Dec. 20, 2012	Patent and licensing status undisclosed	Kallifidas, D. <i>et al.</i> <i>J. Am. Chem. Soc.</i> ; published online Nov. 16, 2012; doi:10.1021/ja3093828 Contact: Sean F. Brady, The Rockefeller University, New York, N.Y. e-mail: sbrady@rockefeller.edu
Multiplexed, microfluidic protease activity assay for biomarker detection	A multiplexed, microfluidic assay that measures protease activity could be used to detect biomarkers. The device contains protease ligands and inhibitors in droplets on a chip that are mixed with picoliter volumes of biological fluids and analyzed using fluorescence resonance energy transfer (FRET)-based proteolytic activity matrix analysis (PrAMA). As proof of concept, the platform analyzed 20 µL samples of peritoneal fluid from patients with endometriosis and healthy controls and identified protease activity profiles that could discriminate between the two groups. Next steps include testing additional clinical samples and further optimizing the detection system. SciBX 5(49); doi:10.1038/scibx.2012.1294 Published online Dec. 20, 2012	Patent application filed; available for licensing	Chen, C.-H. <i>et al.</i> <i>J. Am. Chem. Soc.</i> ; published online Nov. 18, 2012; doi:10.1021/ja307866z Contact: Jongyoon Han, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: jyhan@mit.edu
Scalable, automated microfluidic western blotting	A scalable, automated microfluidics system could help improve the efficiency of western blots. The automated system is compatible with 48-blot microchips and completes western blots in 10–60 minutes while consuming 1,000-fold less antibody than conventional western blots. As proof of concept, the system measured levels of NF-κB in cell lysates and detected HIV proteins in samples. Researchers did not disclose next steps, which could include using the system to detect and quantify levels of disease-linked proteins in clinical samples. SciBX 5(49); doi:10.1038/scibx.2012.1295 Published online Dec. 20, 2012	Patent and licensing undisclosed	Hughes, A.J. & Herr, A.E. <i>Proc. Natl. Acad. Sci. USA</i> ; published online Dec. 5, 2012; doi:10.1073/pnas.1207754110 Contact: Amy E. Herr, University of California, Berkeley, Calif. e-mail: aeh@berkeley.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Drug platforms			
Crystal structure to guide the design of protease-activated receptor 1 (PAR1) antagonists	<p>The crystal structure of human PAR1 bound to vorapaxar could be used to guide the development of improved PAR1 antagonists. In a Phase III trial, the PAR1 antagonist vorapaxar protected patients from myocardial infarction (MI) but led to increased bleeding, which was attributed to the drug's very tight binding. The vorapaxar-bound human PAR1 crystal structure revealed an unusual mode of drug binding that explained the high-affinity interaction that inhibits PAR1 activation. Next steps include collaborations to identify new PAR1 antagonists based on the PAR1-vorapaxar structure.</p> <p>In 2011, Merck & Co. Inc. discontinued dosing of patients in two Phase III trials of vorapaxar to prevent cardiovascular events because intracranial hemorrhage occurred in a subset of patients. In 2012, the pharma announced plans to file regulatory applications for the compound in the same indication but in a restricted patient population.</p> <p>SciBX 5(49); doi:10.1038/scibx.2012.1296 Published online Dec. 20, 2012</p>	Unpatented; licensing status not applicable	<p>Zhang, C. <i>et al. Nature</i>; published online Dec. 9, 2012; doi:10.1038/nature11701</p> <p>Contact: Brian K. Kobilka, Stanford University, Stanford, Calif. e-mail: kobilka@stanford.edu</p>
mRNA vaccines to prevent influenza A infection	<p>Vaccination with a mixture of protamine-complexed mRNAs could help prevent influenza A infection. A mixture of protamine-complexed mRNA was formulated that could be stored for three weeks without loss of efficacy. In both newborn and aged mice, immunization with a vaccine containing influenza A virus hemagglutinin and influenza A virus neuraminidase mRNAs resulted in 100% survival against a lethal influenza virus challenge and no signs of infection, whereas immunization with a control antigen led to 0% survival. In pigs, immunization with a related mRNA vaccine that also contained influenza A virus nucleoprotein (NP) mRNA prevented signs of clinical disease following influenza virus challenge, whereas immunization with a control antigen did not. Next steps include studying the long-term protection conferred by this vaccine approach.</p> <p>CureVac GmbH has mRNA-based vaccines in preclinical development to prevent undisclosed infections (<i>see Translating mRNA vaccines, page 1</i>).</p> <p>SciBX 5(49); doi:10.1038/scibx.2012.1297 Published online Dec. 20, 2012</p>	Patented; Sanofi has an exclusive option to develop vaccines for predefined, undisclosed pathogens; additional indications available for partnering	<p>Petsch, B. <i>et al. Nat. Biotechnol.</i>; published online Nov. 25, 2012; doi:10.1038/nbt.2436</p> <p>Contact: Lothar Stitz, Friedrich Loeffler Institute, Greifswald-Insel Riems, Germany e-mail: lothar.stitz@fli.bund.de</p> <p>Contact: Karl-Josef Kallen, CureVac GmbH, Tubingen, Germany e-mail: josef.kallen@curevac.com</p>

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