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Taming T cells in T1D

By Lev Osherovich, Senior Writer

Separate teams at the **University of California, San Francisco** and **Pfizer Inc.** have evidence that blocking IL-7 signaling in mice can arrest the autoimmune activity behind type 1 diabetes.^{1,2} Whether the findings will translate to the clinic depends on whether the cytokine has the same effects on helper T cell activity in mice and humans.

IL-7 is a key regulator of helper T cells, which ordinarily orchestrate the immune response to foreign antigens. In 2007, researchers at the **Karolinska Institute** found evidence that IL-7 also plays a role in autoimmune disease. The group uncovered human genetic variants in *IL-7 receptor (IL-7R; CD127)* that increased the risk of multiple sclerosis (MS) compared with wild-type IL-7R.³

Also in 2007, researchers at the **University of Cambridge** linked these variants to type 1 diabetes (T1D).⁴

“A lot is known about IL-7 in maintaining T cell homeostasis,” said John Lin, leader of the Pfizer team and VP of experimental medicine at Pfizer’s **Rinat Neuroscience Corp.** unit. “When lymphocyte numbers are really low, IL-7 can restore T cell levels. Its genetic association with autoimmune disease led people to start to look at alterations in IL-7 signaling” as a potential therapeutic target.

Indeed, the Pfizer team reported last year that an antibody against IL-7r reduced disease severity in a mouse model of MS.⁵

“The idea is that if you block IL-7 you’ll block the development and survival of T cells” that are causing an aberrant response to self antigens in autoimmune disease, said UCSF team leader Hans Doms, who has since become an assistant professor of medicine at the **Boston University School of Medicine**.

Repressed memory

The Pfizer and UCSF teams pursued a similar approach in diabetes. In a nonobese diabetic (NOD) mouse model, animals were infused weekly with anti-IL-7r mAbs or a nonspecific antibody and monitored for signs of autoimmunity. Whereas mice given the control mAb spontaneously developed type 1 disease, mice receiving the anti-IL-7r mAbs remained healthy.

The anti-IL-7r antibodies decreased levels of proinflammatory cytokines linked to type 1 diabetes progression compared with control antibody.

“A lot of people are investing in creating new islets, but if you don’t stop the autoimmune response those cells will be destroyed again.”

—Hans Doms,
Boston University
School of Medicine

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Mice on anti-IL-7r immunotherapy also had higher insulin secretion and lower levels of T cell activity in lymph nodes and pancreatic islets.

In mice already beginning to lose β cell mass to autoimmune attack, the anti-IL-7r therapy led to better glycemic control in about 50% of these mice compared with control antibody.

Histopathological analysis of mice with the disease revealed immune cells were present—but quiescent—in pancreatic islets. This suggested that anti-IL-7R treatment caused a shutdown of immune cell activity within the islets, allowing the remaining pancreatic islet β cells to resume functioning.

Altogether, the findings suggest a model in which blockade of IL-7 signaling by anti-IL-7R mAbs reduces both T cell activation and memory activity of cells that already are active (see Figure 1, “Blocking IL-7 in type 1 diabetes”).

Results were published in the *Proceedings of the National Academy of Sciences*. Dooms did not patent his findings. Pfizer has filed patents on its antibodies, and the IP is not available for licensing.

Dooms said sustained activity by memory T cells is needed for type 1 diabetes progression. As a result, he said, blocking the activity of memory cells with anti-IL-7R antibodies might halt the course of the disease—even if considerable damage already has been done.

“Type 1 diabetes is perpetuated by a memory response, and diagnosis occurs late in the game when memory response has already arisen,” said Dooms. “When you go hyperglycemic, you still have about 10%–20% of the islet cell mass left,” enough to sustain insulin production if the memory response can be halted.

Intervening with anti-IL-7R therapy in newly diagnosed patients might “preserve the islet mass that remains by eliminating memory cell activity,” he noted.

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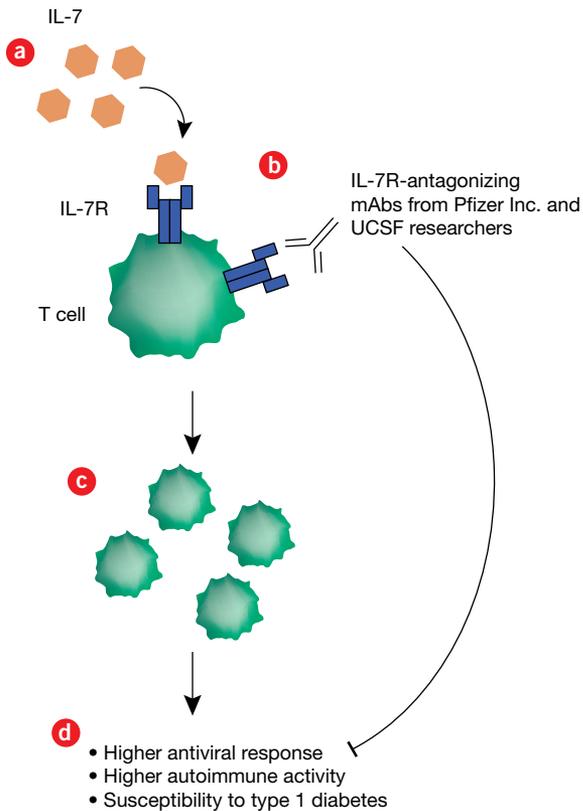


Figure 1. Blocking IL-7 in type 1 diabetes. Lee *et al.* and Penaranda *et al.* have evidence that blocking IL-7 signaling can protect against type 1 diabetes.

IL-7 [a] normally engages the T cell membrane-associated IL-7 receptor (IL-7R; CD127) [b], leading to T cell proliferation and activity [c]. High T cell levels due to IL-7 signaling lead to potent immune activity against pathogens, but mutations thought to activate this pathway are linked with autoimmune disease [d]. Inhibitory IL-7R mAbs independently discovered by **Pfizer Inc.** and by researchers at the **University of California, San Francisco** block IL-7 signaling and arrest the progression of type 1 diabetes in mice. **Effimune S.A.S.** has the anti-IL-7R mAb MD707 in preclinical development for transplant rejection and a range of autoimmune indications.

For patients with advanced disease, Dooks thinks anti-IL-7R treatment could complement regenerative therapies that aim to restore β cell levels.

“A lot of people are investing in creating new islets, but if you don’t stop the autoimmune response those cells will be destroyed again,” he said.

Therapeutic prospects

The teams are now independently working out the mechanism of action of their IL-7R-blocking mAbs.

Dooks and Lin think anti-IL-7R treatment is likely to alter the balance of stimulatory and inhibitory activity in T cells.

Lin’s team found that T cells harvested from mice treated with anti-IL-7r mAbs had higher levels of Pd-1 receptor (Pdc1; Pd-1; Cd279)

than cells from mice given control mAbs. Pd-1 inhibits T cell activity.

Indeed, treatment of diabetic mice with an anti-Pd-1 mAb blocked the beneficial effects of the anti-IL-7r antibodies. What remains less clear is how exactly IL-7 signaling goes awry in type 1 diabetes and whether modulating the pathway might have long-term consequences.

David Booth, associate professor of immunogenetics at **The University of Sydney**, said prior mouse genetic studies suggest eliminating IL-7 activity outright could lead to lymphopenia.

“Inhibiting normal T cell functioning is a concern, given what happens when you knock out the IL-7 receptor,” said Booth. “Knocking it down [with mAbs] could have very unpredictable results” depending on the potency of the anti-IL-7R knockdown.

“The biggest challenge and caveat is the immunosuppressive effect” of blocking IL-7 signaling, agreed Dooks. “To address this concern, our next step is to infect these animals with pathogens to see how immunosuppressed they really are.”

Another question is whether IL-7 and its receptor have the same effects in mice and humans. Michael Demetriou, director of the National Multiple Sclerosis Society Center for MS Comprehensive Care at the **University of California, Irvine**, said the biology of IL-7 signaling is quite complex and is likely to differ between the two species.

“We’ve looked at IL-7 in humans, and it does different things depending on what cell and what stage of immune cell development you’re looking at,” said Demetriou. “In early stages of T cells, it enhances growth and development, but in mature T cells it actually inhibits growth. It’s not as simple as in mice, where if you knock IL-7 out you get lymphopenia and if you agonize it you get autoimmunity.”

“I would be hesitant to put these mAbs into clinical trials until we get a better understanding of the differences between the human and mouse IL-7 systems,” he added.

Indeed, Demetriou and Booth noted that in humans, the autoimmune disease-linked allelic variant of *IL-7R* encodes a protein that is likely to have lower activity than the wild-type form. Thus, it is possible that further lowering IL-7 signaling with antibodies against its receptor could worsen type 1 diabetes, not reverse it.

Lin said this possibility was merely speculative, noting that it is not yet known how the autoimmunity-associated IL-7R variant affects IL-7 signaling.

Angela Crawley, associate scientist at **Ottawa Hospital Research Institute**, said the best-case scenario for the anti-IL-7R strategy is that blocking the receptor would lead to a fairly subtle recalibration of the T cell response away from self antigens without perturbing other immune functions.

By blocking IL-7R, “it may be that you’ve raised the threshold for self antigens but not for pathogen-derived antigens,” she said.

She added that other cytokines such as IL-15 and thymic stromal lymphopoietin (TSLP) also are thought to promote T cell memory response. Thus, she wanted to know how blocking those molecules combines with IL-7R blockade in diabetic mice.

Crawley added that the mouse data in the two papers helps build a case for trying the strategy in the clinic.

“If your goal is to prevent further destruction of the islets, this target should be high on your list,” she said.

The UCSF and Pfizer teams’ findings lend support to the anti-IL-7R strategy being pursued by **Effimune S.A.S.**

Chairman Maryvonne Hiance said the two teams' findings are in line with her company's data for MD707, the company's anti-IL-7R mAb, in preclinical models of other autoimmune indications.

"These two reports provide a strong scientific rationale and significance for anti-IL-7R immunotherapy as a unique strategy for patients with type 1 diabetes," said Hiance. "Very few strategies have been described to reverse established type 1 diabetes in NOD mice."

This month, Effimune reported positive preclinical data for MD707 in a mouse model of transplant.

Hiance said Effimune filed patents in 2011 on using anti-IL-7R mAbs to prevent transplant rejection and to treat a range of autoimmune conditions including type 1 diabetes, lupus and Crohn's disease.

A Pfizer spokesperson declined to disclose the development status of the mAbs described in Lin's study.

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REFERENCES

1. Penaranda, C. *et al. Proc. Natl. Acad. Sci. USA*; published online June 25, 2012; doi:10.1073/pnas.1203692109

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2. Lee, L.-F. *et al. Proc. Natl. Acad. Sci. USA*; published online June 25, 2012; doi:10.1073/pnas.1203795109

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3. Lundmark, F. *et al. Nat. Genet.* **39**, 1108–1113 (2007)
4. Todd, J.A. *et al. Nat. Genet.* **39**, 857–864 (2007)
5. Lee, L.-F. *et al. Sci. Transl. Med.* **3**, 93ra68 (2011)

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Biogen interacts with target discovery

By *Tim Fulmer, Senior Writer*

Biogen Idec Inc. is taking advantage of advances in the study of protein-protein interactions to generate the first comprehensive human interactome. In collaboration with **Harvard Medical School** researchers, the company will map the interaction network of 10,000–15,000 human proteins over the next 4 years, which could provide Biogen with a wealth of new targets in immunological and neurological diseases.

The move is part of Biogen's decision to reignite its target-discovery efforts.¹

"We will initially focus on 200 proteins that we believe are important in neurological and immunological diseases, our key focus areas, and will then expand from there," Biogen EVP of R&D Douglas Williams told *SciBX*.

The goal is "to make Biogen an innovator in defining new pathways and targets. Whereas before we were learning about new targets by reading papers, we now want to get out ahead and be the ones publishing those papers," he said.

Those new targets will "potentially lead to first-in-class drugs, which strengthen our pipeline and make it more competitive," Williams added.

Although the proteome is defined as the set of all proteins expressed by the genome of an organism, the interactome is the set of all interactions between those proteins. Because protein-protein interactions drive almost all cellular functions, complete knowledge of an organism's interactome could provide drug developers with an exhaustive list of potential therapeutic targets.

Over the last decade many academic labs have mapped out interactomes in a variety of small organisms, including yeast² and *Caenorhabditis elegans*.³ The majority of those studies relied on the yeast two-hybrid approach, which measures interactions between any two prespecified proteins.⁴

More recently, mass spectrometry-based approaches to interactome mapping have gained popularity and been used to map the interactomes of yeast,⁵ *Escherichia coli*⁶ and *Mycoplasma pneumoniae*.⁷ However, no one has yet published a comprehensive map of the human interactome using either approach.

To generate such a map, Biogen has set up a collaboration with Steven Gygi and J. Wade Harper at Harvard Medical School, two researchers who have developed a mass spectrometry-based platform to study protein-protein interactions. Gygi is associate professor of cell biology. Harper is professor of cell biology and molecular pathology.

In prior papers published in *Cell* in 2009 and *Nature* in 2010, the researchers used their method to map portions of the human interactome, including the ubiquitinating enzyme interaction network and the autophagy interaction network.^{8,9}

"We believe the Harvard team's platform is the most accurate out there for identifying protein-protein interactions, and that's why we chose it to generate our human interactome," Biogen CSO Spyros Artavanis-Tsakonas told *SciBX*.

Artavanis-Tsakonas has firsthand experience with the platform. Prior to joining Biogen in March, Artavanis-Tsakonas collaborated with Gygi to generate a *Drosophila* interactome. The resulting *Drosophila* Protein Interaction Map (DPiM), published in *Cell* in 2011, encompassed 566 protein complexes involved in a variety of cell signaling pathways.¹⁰

"The clear advantage of the mass spec-based approach over the yeast two-hybrid approach is that the former allows you to measure not only interactions between any two proteins but also simultaneous interactions between multiple proteins that form ensembles and complexes in the cell," said Artavanis-Tsakonas. "That is very important because those complexes play a crucial role in cell signaling pathways and are potential drug targets."

As the first step of the program, the Harvard team will generate baseline maps of the human interactome in a number of healthy cell types, including 3T3 cells, which are a standard fibroblast cell line, as well as CNS and immune cells. Next, Biogen and the Harvard researchers will generate interactome maps of neuronal and immune cell lines that model various disease phenotypes.

Finally, Biogen and the Harvard group will use statistical and data analysis to measure differences between the healthy and disease interactome maps. Such differences could be qualitative, such as the absence or presence

of a particular protein-protein interaction or complex, or quantitative, such as decreased or increased levels of a particular interaction or complex.

Generating even a single interactome map of a particular healthy or diseased cell type will be a time-consuming and data-intensive process. Each of the proteins potentially included in the map will have to be expressed in a given cell type, isolated using affinity purification, put through mass spectrometry to identify all of its interaction partners and analyzed using statistical tools to rule out false-positive interactions.

Nonetheless, once the healthy and disease interactome maps are completed, differences between the two maps can be used to quickly identify variations in protein-protein interactions that may signify potential therapeutic targets.

It should then be possible to engineer those variations into animals to characterize them in an *in vivo* setting. Based on the *in vitro* and *in vivo* data, researchers then will be in a position to decide how best to target a particular interaction in a given disease.

Harvard will own any IP resulting from the Interactome Program. Biogen Idec will have the option to negotiate exclusive rights to patentable discoveries. Biogen is providing funding in the single-digit millions.

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"We believe the Harvard team's platform is the most accurate out there for identifying protein-protein interactions, and that's why we chose it to generate our human interactome."

—*Spyros Artavanis-Tsakonas,*
Biogen Idec Inc.

REFERENCES

1. Schaeffer, S. *BioCentury* **20**(27), A10–A11; July 2, 2012
2. Ito, T. *et al. Proc. Natl. Acad. Sci. USA* **98**, 4569–4574 (2001)
3. Li, S. *et al. Science* **303**, 540–543 (2004)
4. Brückner, A. *et al. Int. J. Mol. Sci.* **10**, 2763–2788 (2009)
5. Krogan, N.J. *et al. Nature* **440**, 637–643 (2006)
6. Hu, P. *et al. PLoS Biol.* **7**, e96; published online April 28, 2009; doi:10.1371/journal.pbio.1000096
7. Kühner, S. *et al. Science* **326**, 1235–1240 (2009)
8. Sowa, M.E. *et al. Cell* **138**, 389–403 (2009)
9. Behrends, C. *et al. Nature* **466**, 68–76 (2010)
10. Guruharsha, K.G. *et al. Cell* **147**, 690–703 (2011)

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Target on Ebola

By Tracey Baas, Senior Editor

Public Health Agency of Canada researchers have shown that a cocktail of three antibodies, dubbed ZMab, improved the survival of nonhuman primates infected by Ebola virus.¹ ZMab is licensed to **Defyryus Inc.**, which plans to establish commercial-scale manufacture of the therapy and carry out biodistribution and toxicology studies.

An ongoing challenge for Ebola therapies is the ability to treat the disease several days after infection, when patients typically present at the clinic. Ebola runs its course within 14–21 days, with patients first presenting nonspecific flu-like symptoms. Patients then progress and show impaired kidney and liver function, and in some cases internal and external bleeding.

There are no licensed antiviral therapies or vaccines for the virus, and treatments in development, including anticoagulants, antisense molecules and antibodies, are only effective when given within a few hours of infection.

For the past two years, the Public Health Agency of Canada (PHAC) researchers have been working to widen that treatment window using an antibody-based approach.

In prior work, they showed that each of three murine mAbs targeting the Ebola Zaire glycoproteins GP1 and GP2 and a precursor of GP1 and GP2, called GP, protected mice from Ebola challenge when given 48 hours after infection.² When combined into a cocktail, the 3 mAbs protected guinea pigs from Ebola challenge when given 48 hours after infection.³

Based on those data, the researchers decided to test the three mAbs as a cocktail in cynomolgus macaques.

At 24 hours after a lethal Ebola challenge, 3 doses of ZMab given 3 days apart protected all 4 animals from death. When the cocktail was started 48 hours after lethal challenge, 2 of 4 animals survived. In survivors, the treatment led to lower viral loads, mild symptoms, no shedding and normal blood biochemistry and hematology.

Virus isolated from one of the nonsurviving animals had developed two escape mutations, suggesting the cocktail could trigger resistance.

Results were published in *Science Translational Medicine*.

Mouse vs. human mAb

The PHAC researchers are collaborating with life sciences company Defyryus, which has exclusive rights to manufacture and commercialize ZMab.

Defyryus' lead compound is DEF201, a replication-defective adenovirus vector that encodes the gene for the antiviral human interferon- α (IFNA; IFN- α) protein. The broad-acting antiviral is in preclinical development for multiple disease indications.

Defyryus president and CEO Jeffrey Turner said DEF201 could be used in combination with ZMab. "With a suspected Ebola virus infection, nasal delivery of DEF201 slows disease progression and buys time while diagnostics are performed to confirm an Ebola infection. Upon confirmation of Ebola infection, ZMab would then immediately be given," he said.

Defyryus plans to start clinical trials of murine ZMab within 24 months once GMP manufacturing is established and preclinical safety and toxicology are confirmed.

Gary Kobinger, PHAC team leader, said he is interested in having the murine mAbs humanized but added that "it's not a requirement to move into the clinic." Kobinger is chief of special pathogens, head of vector design and immunotherapy for the Special Pathogens Program at the National Microbiology Laboratory at PHAC and adjunct professor of medical microbiology and immunology at the **University of Manitoba**.

"We hypothesize that a humanized ZMab treatment might have a longer half-life, so we might be able to see protection with only two doses rather than three," he noted. "But our first priority is to move the murine ZMab treatment forward."

Patrick Iversen, SVP of research and innovation at **Sarepta Therapeutics Inc.**, told *SciBX* he would prioritize differently. "Humanizing the mAb is a critical next step because clinical studies using mouse mAbs for other indications showed that patients developed antibodies against mouse mAbs," he said.

"Humanizing the mAb is a critical next step because clinical studies using mouse mAbs for other indications showed that patients developed antibodies against mouse mAbs."

—Patrick Iversen,
Sarepta Therapeutics Inc.

Iversen also wanted to see "what type of immune responses the nonhuman primates showed toward the mouse ZMab cocktail. Further preclinical studies will need to include immunotoxicity testing in addition to standard safety pharmacology and repeat dose toxicology testing."

Sarepta's AVI-7537 antisense molecule is in Phase I testing for Ebola.

"I think his concern is legitimate because serum sickness is a real adverse effect when

antibodies are administered in an individual with concomitant anti-antibodies," Kobinger said. "In our case, however, there are only three injections administered within nine days, which is not long enough for anti-antibodies to develop while the mouse antibodies are still being given."

He added, "That being said, we have already humanized the 3 monoclonals and hope to produce and test them within the next 12 months or so. In the meantime, the mouse monoclonals do work with no apparent side effect in nonhuman primates. Considering the probable 100% mortality of an accidental lab-acquired Ebola virus infection and what is available out there, I myself wouldn't worry too much about antimouse antibodies when deciding on the course of action would I be accidentally exposed to Ebola virus?"

Also, deep sequencing methods will be needed to better understand potential mechanisms of emerging viral resistance, said Iversen. "The entire viral genome must be evaluated for resistance mutations in all infected animals, not just mutations in the GP gene and not just in euthanized nonsurvivors."

The group's ultimate goal "is a treatment or combination of treatments to push past the barrier of 72 hours after infection," said Kobinger. The team also wants to develop other antibody-based strategies to treat similar diseases, such as Lassa, Rift Valley and Crimean-Congo hemorrhagic fevers.

A patent application covering the paper's findings has been filed by PHAC and is exclusively licensed to Defyryus.

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(Continues on p. 8)

Crafty air carrier

By Michael J. Haas, Senior Writer

A team led by Boston-area researchers has shown that infusion of oxygen-loaded microparticles could keep the blood of asphyxiated rabbits oxygenated, preventing organ damage and death.¹ Although the method could help treat hypoxemia resulting from acute airway obstruction or damage, future studies will have to show that the microparticles are safe and have the biochemical properties of endogenous hemoglobin.

Current strategies for treating hypoxemia include insertion of a breathing tube through the throat and mechanical ventilation. However, those procedures can take several minutes to implement, and even a few minutes of hypoxemia can cause lactic acid buildup (lactic acidosis), which triggers tachycardia, seizures, coma, brain and other organ damage, cardiac arrest or death.

Therapeutics to treat hypoxemia include oxygen-absorbing perfluorocarbons such as perflubron² as well as human hemoglobin-based carriers such as Sangart Inc.'s MP4OX. MP4OX is an oxygenated, pegylated, hemoglobin-based colloid that is in Phase IIb testing to treat lactic acidosis caused by hemorrhagic shock in severely injured trauma patients.

However, perfluorocarbon- and hemoglobin-based carriers must be reoxygenated by circulation through the lungs and thus are ineffective when normal respiration has been compromised by tracheal damage or airway blockage.

The Boston team was inspired to develop a new, rapid method to treat hypoxemia by the experience of team leader John Kheir when he treated a patient with pneumonia.

When the patient experienced an abrupt onset of hypoxemia as a result of pneumonia complications, Kheir inserted a breathing tube and placed her on a heart-lung bypass machine. But during the 15–20 minute period required for these procedures, her hypoxemia caused “profound brain injury, and she subsequently died,” Kheir told *SciBX*.

Frustrated by the lack of technologies for rapid treatment of hypoxemia, “I formed a team with the capabilities of creating a solution to this problem by manufacturing oxygen gas-filled microparticles,” he said.

Kheir is a staff physician in the Cardiac Intensive Care Unit at **Boston Children's Hospital** and an instructor of pediatrics at **Harvard Medical School**.

Kheir's team began by developing microparticles composed of a phospholipid-copolymer shell loaded with free oxygen and formulated in Plasma-Lyte—an i.v. fluid similar to plasma. The suspensions contained up to 90% oxygen by volume, remained stable at 4 °C for up to 100 days and released their cargo when exposed to a low-oxygen environment such as that found in venous blood.

Although 90% oxygen-by-volume suspensions would carry the maximum possible payload, the team found any suspension containing more than 70% oxygen by volume did not adequately mix with whole human blood, thereby slowing the subsequent transfer of oxygen cargo to hemoglobin.

In *ex vivo* deoxygenated human red blood cells, 70% by-volume suspensions delivered their oxygen cargo and reoxygenated hemoglobin within 4 seconds compared with 71 seconds for 90% by-volume suspensions.

Thus, the team chose suspensions containing 70% oxygen by volume for the rest of the experiments, including studies of the microparticles in normal rabbits that had moderate hypoxemia induced by ventilation on low-oxygen air.

The group infused the hypoxemic rabbits with microparticles for two minutes, during which time the fraction of arterial hemoglobin that was oxygenated rose from 65%–70% to near-normal levels of more than 90%. The microparticles also reversed the hypoxemia-induced increase in blood pressure in the rabbits.

Finally, the team tested the microparticles in a rabbit model of airway obstruction-induced asphyxia, in which a surgically implanted tracheal cuff was clamped shut to cut off the supply of inhaled oxygen. In this model, continuous infusion of microparticles during 15 minutes of asphyxiation normalized blood levels of oxyhemoglobin and decreased acidosis, signs of liver injury and the incidence of cardiac arrest and increased survival compared with free vehicle infusion.

The treatment caused no observable complications or side effects, such as pulmonary embolisms, tachycardia or hypotension.

The results demonstrate that the technology could provide rapid, short-term oxygenation to patients with acute airway obstruction or lung failure, or during lengthy intubation procedures, to prevent organ damage and cardiac arrest, the team wrote in its report in *Science Translational Medicine*.¹

The group included researchers from **Columbia University**, **Harvard University**, the **Medical University of South Carolina**, the **University of Colorado at Boulder** and formulation CRO **Particle Sciences Inc.**

Airing differences

For the technology to clear clinical and regulatory hurdles, future studies should show that the microparticles can deliver oxygen and

(Continues on p. 9)

(Continued from “Target on Ebola,” p. 7)

REFERENCES

1. Qiu, X. *et al. Sci. Transl. Med.*; published online June 13, 2012; doi:10.1126/scitranslmed.3003876
Contact: Gary P. Kobinger, University of Manitoba, Winnipeg, Manitoba, Canada
e-mail: gary.kobinger@phac-aspc.gc.ca
2. Qiu, X. *et al. Clin. Immunol.* **141**, 218–227 (2011)

3. Qiu, X. *et al. PLoS Negl. Trop. Dis.* **6**, e1575; published online March 20, 2012; doi:10.1371/journal.pntd.0001575

COMPANIES AND INSTITUTIONS MENTIONED

Defyrus Inc., Toronto, Ontario, Canada
Public Health Agency of Canada, Winnipeg, Manitoba, Canada
Sarepta Therapeutics Inc. (NASDAQ:SRPT), Bothell, Wash.
University of Manitoba, Winnipeg, Manitoba, Canada

maintain blood pressure in the way endogenous hemoglobin does, said David Platt, chairman, director, CEO and CFO of **Boston Therapeutics Inc.**

Indeed, the blood-substitute space is littered with failed hemoglobin-based products such as PolyHeme from now-defunct Northfield Laboratories Inc., HemAssist from **Baxter International Inc.** and Optro rHb1.1 from Somatogen Inc., now part of Baxter.

“To date, the FDA has not approved any blood substitutes or artificial oxygen carriers because they do not have all of the critical attributes of hemoglobin” contained in red blood cells, Platt said. Among those attributes are the ability to deliver about 250 mL/min of oxygen—the normal amount carried by arterial blood in a healthy person—and the ability to scavenge the right amount of nitric oxide (NO) required to maintain proper blood pressure, he said.

“Kheir’s team will need to show their microparticles meet these two criteria or they don’t have any chance of clinical success,” Platt said.

Boston Therapeutics’ Ipoxyn, a stabilized glycoprotein containing oxygen-rechargeable iron, is in preclinical testing to treat hypoxia.

Kheir said the microparticles can deliver the amount of oxygen that a human requires. He noted that in the rabbit models of asphyxia, the microparticles delivered 4 mL/min of oxygen per kg of body weight—the equivalent of 200–250 mL/min in an average adult human.

Furthermore, “a major advantage of the microparticles is that they do not interact with the nitric oxide system” and thus do not affect blood pressure by scavenging too much or too little NO, he said.

Both Platt and Jennifer Johnson, cofounder and COO of **NuvOx Pharma LLC**, said the size of the microparticles presented a potential safety problem.

Johnson noted that the Kheir team’s microparticles averaged about 4 μm in diameter—about half the size of red blood cells—but a small fraction of the particles were 10 μm or larger. Particles of that size “could block capillaries and cause stroke, pulmonary embolism or heart attack,” she said. “It is not likely the FDA would approve particles with such a wide distribution in size” because of those safety issues.

Evan Unger, cofounder, president and CEO of NuvOx, suggested testing the safety of the microparticles in dogs, which “tend to be much more sensitive than other species to the size distribution of intravenous microparticle formulations.”

NuvOx’s lead compound, NVX-108, a submicroemulsion of the perfluorocarbon dodecafluoropentane (DDFP), is in preclinical testing to treat fetal hypoxia, nitrogen narcosis, stroke-related hypoxic brain damage and other indications involving hypoxia.

Platt also noted that platelets and artificial oxygen carriers such as perfluorocarbons can form aggregates that cause blood clots or deposit in the tissues with long-term toxicity issues. He wanted to know where in the body the microparticles go and how long they take to clear.

“Until the longevity of the breakdown products of the microparticles are known, it is difficult to suggest a use for them, other than a very short-lived, short-term intervention,” he said.

Johnson agreed. Assuming the team could reduce and control the microparticles’ size, “they would seem best suited for short-term oxygenation when the patient is unable to breathe.”

Added Unger, “They seem least suited for treatment longer than about 15 minutes in a nonbreathing patient, because this would require multiple doses and would not prevent the accumulation of carbon dioxide or help remove it.”

Kheir said his team is running safety studies to determine the *in vivo* clearance of the spent or degraded microparticles. The team also is testing the oxygen-loaded microparticles in animal models of cardiac arrest, he said.

He added that the technology could be used to deliver other gases to the bloodstream for therapeutic or diagnostic purposes but declined to describe specific applications.

The findings reported in the paper are patented. The team plans to develop the microparticles through Phase I and then seek a partner or licensing deal.

Haas, M.J. *SciBX* 5(29); doi:10.1038/scibx.2012.749

Published online July 26, 2012

REFERENCES

1. Kheir, J.N. *et al. Sci. Transl. Med.*; published online June 27, 2012; doi:10.1126/scitranslmed.3003679
Contact: John N. Kheir, Boston Children’s Hospital, Boston, Mass. e-mail: john.kheir@childrens.harvard.edu
2. Spahn, D.R. *et al. Anesthesiology* 91, 1195–1208 (1999)

COMPANIES AND INSTITUTIONS MENTIONED

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Boston Children’s Hospital, Boston, Mass.
Boston Therapeutics Inc. (OTCBB:BTHE), Manchester, N.H.
Columbia University, New York, N.Y.
Harvard Medical School, Boston, Mass.
Harvard University, Cambridge, Mass.
Medical University of South Carolina, Charleston, S.C.
NuvOx Pharma LLC, Tucson, Ariz.
Particle Sciences Inc., Bethlehem, Pa.
Sangart Inc., San Diego, Calif.
University of Colorado at Boulder, Boulder, Colo.

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)	Potassium channel KIR4.1 (KCNJ10)	Human studies suggest detecting serum antibodies against KCNJ10 could help diagnose MS. Immunoreactive assays of human serum identified anti-KCNJ10 antibodies in 47% of patients with MS compared with less than 1% of patients with other neurological diseases and 0% of healthy controls. Ongoing work includes optimizing the assay and conducting prospective studies in a larger population of patients with MS to evaluate anti-KCNJ10 antibodies as a marker of treatment response.	Patented; available for licensing or partnering	Srivastava, R. <i>et al. N. Engl. J. Med.</i> ; published online July 12, 2012; doi:10.1056/NEJMoa1110740 Contact: Bernhard Hemmer, Technical University Munich, Munich, Germany e-mail: hemmer@lrz.tu-muenchen.de
SciBX 5(29); doi:10.1038/scibx.2012.750 Published online July 26, 2012				
Cancer				
Breast cancer	Basic helix-loop-helix family member e41 (BHLHE41; BHLHB3; DEC2)	Patient sample and mouse studies suggest BHLHE41 could help treat and diagnose triple-negative breast cancer. In primary triple-negative breast cancer samples, high BHLHE41 expression was correlated with increased survival and reduced probability of metastasis. In mice injected with metastatic breast cancer cells, low BHLHE41 expression led to metastasis to the lung, whereas BHLHE41 overexpression did not. Next steps include identifying a compound that upregulates BHLHE41 and evaluating the prognostic value of BHLHE41 expression in larger patient cohorts.	Diagnostic applications of BHLHE41 in breast cancer patented; licensed to an undisclosed company for development of a diagnostic kit	Montagner, M. <i>et al. Nature</i> ; published online July 8, 2012; doi:10.1038/nature11207 Contact: Stefano Piccolo, University of Padua School of Medicine, Padua, Italy e-mail: piccolo@bio.unipd.it
SciBX 5(29); doi:10.1038/scibx.2012.751 Published online July 26, 2012				
Cancer	DNA	Cell culture studies suggest the DNA-binding platinum(II) complex phenanthriplatin could help treat cancer. In a panel of seven human cancer cell lines, phenanthriplatin inhibited growth with greater potency than cisplatin, oxaliplatin or pyriplatin. In a panel of 60 human cancer cell lines, phenanthriplatin showed a different spectrum of inhibitory activity than conventional platinum-based crosslinking agents. Next steps include evaluating phenanthriplatin in <i>in vivo</i> efficacy studies and testing it in combination with nanoparticle carrier molecules. Cisplatin and oxaliplatin are generic, platinum-based drugs for cancer. Pyriplatin is a platinum(II) complex-based research reagent.	Patent application filed; licensing status undisclosed	Park, G.Y. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 6, 2012; doi:10.1073/pnas.1207670109 Contact: Stephen J. Lippard, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: lippard@mit.edu
SciBX 5(29); doi:10.1038/scibx.2012.752 Published online July 26, 2012				

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Lung cancer	B cell lymphoma 2 (BCL-2; BCL2); Bcl-x _L	<p><i>In vitro</i> and mouse studies suggest a class of dual BCL2 and Bcl-x_L inhibitors could help treat lung cancer. Chemical synthesis, SAR studies and <i>in vitro</i> testing of 4,5-diphenyl-1H-pyrrole-3-carboxylic acid analogs identified several lead compounds as low nanomolar dual BCL2 and Bcl-x_L inhibitors. In a human small cell lung cancer cell line, the lead compounds induced dose-dependent apoptosis. In mice with xenograft small cell lung cancer tumors, one lead compound decreased tumor growth compared with vehicle without significant toxicity. Future studies could include testing the lead compounds in animal models of other cancers.</p> <p>AT-101, a small molecule pan-inhibitor of BCL-family proteins from Ascenta Therapeutics Inc. and Ascentage Pharma Group Corp. Ltd., is in Phase II testing to treat prostate and brain cancers, non-small cell lung cancer (NSCLC), chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphoma (NHL). It is also in Phase I/II testing to treat small cell lung cancer and esophageal cancer.</p> <p>Obatoclox (CEP-41601; GX15-070), a small molecule BCL2 antagonist from Teva Pharmaceutical Industries Ltd., is in Phase II testing to treat small cell lung cancer.</p> <p>Navitoclax (ABT-263; RG7433), a pan-inhibitor of BCL2-family proteins from Abbott Laboratories and Roche's Genentech Inc. unit, is in Phase I/II testing to treat small cell lung cancer.</p> <p>SciBX 5(29); doi:10.1038/scibx.2012.753 Published online July 26, 2012</p>	Patent and licensing status unavailable	<p>Zhou, H. <i>et al. J. Med. Chem.</i>; published online July 2, 2012; doi:10.1021/jm300608w Contact: Shaomeng Wang, University of Michigan, Ann Arbor, Mich. e-mail: shaomeng@umich.edu Contact: Jeanne A. Stuckey, same affiliation as above e-mail: jass@umich.edu</p>
Melanoma	Fibroblast growth factor receptor 3 (FGFR3; CD333)	<p>Cell culture studies suggest inhibiting FGFR3 could help sensitize melanomas to BRAF inhibitors. BRAF V600E mutant human melanoma cell lines that have acquired resistance to the BRAF inhibitor Zelboraf vemurafenib showed activation of the MEK-MAP kinase (ERK) pathway. In the Zelboraf-resistant cells, small interfering RNA or an inhibitor against FGFR3 prevented MEK-ERK pathway activation and restored Zelboraf sensitivity, and decreased cancer cell proliferation compared with control siRNA or vehicle. Next steps could include testing FGFR3 inhibition in additional models.</p> <p>Roche, Chugai Pharmaceutical Co. Ltd. and Daiichi Sankyo Co. Ltd. market Zelboraf to treat metastatic melanoma.</p> <p>SciBX 5(29); doi:10.1038/scibx.2012.754 Published online July 26, 2012</p>	Patent and licensing status unavailable	<p>Yadav, V. <i>et al. J. Biol. Chem.</i>; published online June 22, 2012; doi:10.1074/jbc.M112.377218 Contact: Sheng-Bin Peng, Eli Lilly and Co., Indianapolis, Ind. e-mail: sbpeng@lilly.com</p>
Melanoma	IL-9	<p>Patient sample and mouse studies suggest IL-9 could help treat melanoma. In mouse models of melanoma and Lewis cell carcinoma, recombinant IL-9 inhibited tumor growth. In samples from patients with metastatic melanoma, levels of T helper type 9 cells that secrete IL-9 were lower than those in samples from healthy human skin and blood. Next steps could include testing recombinant IL-9 in additional animal models of cancer.</p> <p>SciBX 5(29); doi:10.1038/scibx.2012.755 Published online July 26, 2012</p>	Patent and licensing status unavailable	<p>Purwar, R. <i>et al. Nat. Med.</i>; published online July 8, 2012; doi:10.1038/nm.2856 Contact: Thomas S. Kupper, Harvard Medical School, Boston, Mass. e-mail: tkupper@partners.org or tskupper@rics.bwh.harvard.edu</p>

This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Endocrine/metabolic disease				
Diabetes	Cryptochrome 1 (CRY1)	Cell culture studies identified a small molecule circadian rhythm regulator that could help treat diabetes. In a cell-based screen of about 60,000 compounds, a CRY1-stabilizing molecule lengthened the circadian period in human osteosarcoma cell lines, mouse fibroblasts and mouse lung explants. In mouse primary hepatocytes, the compound inhibited glucagon (GCG)-mediated activation of glucose production without altering basal glucose production. Next steps include developing circadian rhythm-regulating research compounds for use in <i>in vivo</i> studies. SciBX 5(29); doi:10.1038/scibx.2012.756 Published online July 26, 2012	Unpatented; licensing status not applicable	Hirota, T. <i>et al. Science</i> ; published online July 12, 2012; doi:10.1126/science.1223710 Contact: Steve A. Kay, University of California, San Diego, La Jolla, Calif. e-mail: skay@ucsd.edu Contact: Peter G. Schultz, The Scripps Research Institute, La Jolla, Calif. e-mail: schultz@scripps.edu
Diabetes	IL-7 receptor (IL-7R; CD127)	Two studies in mice suggest antagonizing CD127 could help treat type 1 diabetes. In a mouse model of type 1 diabetes, antibodies against CD127 decreased autoimmune T cell activity compared with nonspecific control antibody and prevented disease onset and arrested existing disease. Next steps include preclinical development of humanized anti-CD127 antibodies. Effimune S.A.S.'s MD707, an anti-CD127 antibody, is in preclinical development to prevent transplant rejection (<i>see Taming T cells in T1D, page 1</i>). SciBX 5(29); doi:10.1038/scibx.2012.757 Published online July 26, 2012	Findings in first study unpatented; unlicensed Patent applications filed by Pfizer Inc. for findings in second study; unavailable for licensing	Penaranda, C. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 25, 2012; doi:10.1073/pnas.1203692109 Contact: Hans Dooms, Boston University School of Medicine, Boston, Mass. e-mail: hdooms@bu.edu Lee, L.-F. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 25, 2012; doi:10.1073/pnas.1203795109 Contact: John C. Lin, Pfizer Inc., South San Francisco, Calif. e-mail: john.lin@pfizer.com Contact: Li-Fen Lee, same affiliation as above e-mail: li-fen.lee@pfizer.com
Mucopolysaccharidosis (MPS)	Arylsulfatase G (ARSG)	Mouse and <i>in vitro</i> studies suggest restoring ARSG levels could be useful for treating MPS type IIIE, a lysosomal storage disorder caused by impaired processing of heparan sulfate. Mice lacking Arsg accumulated unprocessed heparan sulfate degradation products and developed neurological abnormalities resembling MPS type IIIE. <i>In vitro</i> , recombinant ARSG degraded heparan sulfate, whereas a nonselective control enzyme did not. Next steps could include formulating a recombinant version of ARSG for preclinical testing. SciBX 5(29); doi:10.1038/scibx.2012.758 Published online July 26, 2012	Patent and licensing status undisclosed	Kowalewski, B. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 11, 2012; doi:10.1073/pnas.1202071109 Contact: Thomas Dierks, Bielefeld University, Bielefeld, Germany e-mail: thomas.dierks@uni-bielefeld.de
Infectious disease				
HIV/AIDS	Protein kinase C (PKC)	<i>In vitro</i> studies suggest a new class of PKC agonists could help treat HIV infection. Chemical synthesis and <i>in vitro</i> testing of bryostatin analogs identified seven compounds as low nanomolar PKC agonists. In a human T cell line model of latent HIV infection, the compounds reactivated latent virus at nanomolar and subnanomolar EC ₅₀ values, which would render the virus sensitive to standard antiretroviral drugs. A forthcoming publication will report the results of testing the compounds in blood samples from patients with HIV who are on antiretroviral therapy and have undetectable serum levels of the virus. SciBX 5(29); doi:10.1038/scibx.2012.759 Published online July 26, 2012	Patented; available for licensing	DeChristopher, B.A. <i>et al. Nat. Chem.</i> ; published online July 15, 2012; doi:10.1038/nchem.1395 Contact: Paul A. Wender, Stanford University, Stanford, Calif. e-mail: wenderp@stanford.edu Contact: Jerome A. Zack, University of California, Los Angeles, Calif. e-mail: jzack@ucla.edu

This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Inflammation				
Inflammation; shock/ trauma	MAP kinase- activated protein kinase 2 (MAPKAPK2; MK2); tumor necrosis factor- α (TNF- α)	<i>In vitro</i> and mouse studies suggest a class of MK2 inhibitors could help treat septic shock and other inflammatory disorders. Library screening, chemical synthesis and <i>in vitro</i> testing of pyrazolo[1,5- <i>a</i>]pyrimidine analogs identified three lead compounds as selective, nanomolar inhibitors of MK2. In a mouse model of lipopolysaccharide (LPS)-induced septic shock, one lead compound decreased proinflammatory serum Tnf- α levels compared with vehicle. Results of undisclosed ongoing studies by Teijin Pharma Ltd. and Galapagos N.V.'s BioFocus plc unit will be reported in a forthcoming publication. MMI-0100, an MK2 inhibitor from Moerae Matrix Inc. and MicroDose Therapeutx Inc., is in preclinical testing to treat inflammation and pulmonary fibrosis. SciBX 5(29); doi:10.1038/scibx.2012.760 Published online July 26, 2012	Patented by Teijin Pharma; unavailable for licensing	Kosugi, T. <i>et al. J. Med. Chem.</i> ; published online July 2, 2012; doi:10.1021/jm300411k Contact: Gen Unoki, Teijin Pharma Ltd., Tokyo, Japan e-mail: g.unoki@teijin.co.jp Contact: Dale Robert Mitchell, GlaxoSmithKline Medicines Research Centre, Stevenage, U.K. e-mail: dale.mitchell@gplg.com
Neurology				
Amyotrophic lateral sclerosis (ALS)	Monocarboxylate transporter 1 (MCT1)	Mouse and patient sample studies suggest increasing MCT1 levels could help decrease neurodegeneration in ALS. In mice, Mct1 knockdown in the optic nerve or corpus callosum increased axon degeneration compared with normal Mct1 expression. In an ALS mouse model and patients with ALS, oligodendrocyte MCT1 levels were lower than those in non-ALS mice and patients. Ongoing work includes developing small molecules and using genetic overexpression to increase MCT1 levels in mouse models of ALS and evaluate disease progression. SciBX 5(29); doi:10.1038/scibx.2012.761 Published online July 26, 2012	Unpatented; available for licensing	Lee, Y. <i>et al. Nature</i> ; published online July 12, 2012; doi:10.1038/nature11314 Contact: Jeffrey D. Rothstein, The Johns Hopkins University, Baltimore, Md. e-mail: jrothstein@jhmi.edu
Depression	NADPH oxidase; neutrophil cytosolic factor 1 (NCF1; p47phox)	<i>In vitro</i> and mouse studies suggest inhibiting NADPH oxidase or its NCF1 subunit could help treat depression. In human and mouse neuronal cell lines, the stress hormone glucocorticoid increased NCF1 levels compared with an inactive control compound. In mouse models of restraint-induced stress, depressive behaviors were greater and cortical and hippocampal levels of Ncf1 were higher than those in unrestrained controls. In these models, Ncf1 deficiency or an NADPH oxidase inhibitor decreased depressive behaviors compared with normal Ncf1 expression or an inactive control compound. Next steps could include studying the role of Ncf1 in other animal models of depression. GKT137831, a dual NADPH oxidase 1 (NOX1) and NOX4 inhibitor from Genkyotex S.A., is in Phase I testing to treat diabetic nephropathy and preclinical testing to treat pulmonary fibrosis. SciBX 5(29); doi:10.1038/scibx.2012.762 Published online July 26, 2012	Patent and licensing status unavailable	Seo, J.-S. <i>et al. J. Neurosci.</i> ; published online July 11, 2012; doi:10.1523/JNEUROSCI.0794-12.2012 Contact: Pyung-Lim Han, Ewha Womans University, Seoul, South Korea e-mail: plhan@ewha.ac.kr

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Pain; depression	Indoleamine 2,3-dioxygenase 1 (IDO1)	Rat studies suggest inhibiting IDO1 could help treat pain and depression. Arthritic rats showing pain-induced depressive behaviors had higher hippocampal Ido1 levels than nonarthritic rats. In the same rats, hippocampal injection of the IDO1 inhibitor 1-methyl-D-tryptophan (D-1MT) decreased nociceptive and depressive behaviors compared with vehicle injection. Next steps include optimizing the dosing of an IDO inhibitor in animal models. NewLink Genetics Corp.'s D-1MT is in Phase I/II testing to treat solid tumors. INCB24360, an IDO inhibitor from Incyte Corp., is in Phase II testing to treat solid tumors. SciBX 5(29); doi:10.1038/scibx.2012.763 Published online July 26, 2012	Patent application filed covering use in pain and depression; available for licensing from the Massachusetts General Hospital Office of Research Ventures and Licensing	Kim, H. <i>et al. J. Clin. Invest.</i> ; published online July 2, 2012; doi:10.1172/JCI61884 Contact: Jianren Mao, Harvard Medical School, Boston, Mass. e-mail: jmao@partners.org
Psychosis; emotional lability	NMDA receptor NR2A subtype (GRIN2A; NR2A); GRIN2B (NR2B)	Mouse studies suggest NR2A and NR2B antagonists could help treat aggressive behavior. Humans and mice with a monoamine oxidase A (MAO-A) deficiency show aggressive outbursts in response to stress. In <i>Mao-a</i> -deficient mice, NR2A- or NR2B-selective NMDAR antagonists decreased aggression compared with saline. Next steps include dose-ranging studies in animal models. At least 17 companies have NMDAR antagonists on the market or in development to treat neurological and psychiatric indications. SciBX 5(29); doi:10.1038/scibx.2012.764 Published online July 26, 2012	Unpatented; licensing status not applicable	Bortolato, M. <i>et al. J. Neurosci.</i> ; published online June 20, 2012; doi:10.1523/JNEUROSCI.0225-12.2012 Contact: Jean C. Shih, University of Southern California, Los Angeles, Calif. e-mail: jcshih@usc.edu Contact: Marco Bortolato, same affiliation as above e-mail: bortolat@usc.edu
Various				
Colorectal cancer; inflammatory bowel disease (IBD); colitis	Epidermal growth factor receptor (EGFR)	Mouse studies suggest increasing EGFR activity could help treat IBD and colitis without also raising cancer risk. In two mouse models of colitis, inhibition of Egfr signaling increased colitis severity and the incidence and progression of colon tumors compared with normal Egfr signaling. Next steps include testing the effect of activating the EGFR pathway to prevent colitis-associated cancers. At least four companies have EGFR inhibitors in development stages ranging from clinical to marketed to treat colorectal cancer. SciBX 5(29); doi:10.1038/scibx.2012.765 Published online July 26, 2012	Unpatented; licensing status not applicable	Dubé, P.E. <i>et al. J. Clin. Invest.</i> ; published online July 9, 2012; doi:10.1172/JCI62888 Contact: D. Brent Polk, University of Southern California and Children's Hospital Los Angeles, Los Angeles, Calif. e-mail: dbpolk@chla.usc.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			
A detector to quantify antibody- and magnetic nanoparticle-tagged circulating tumor cells (CTCs) in whole blood	A detector that quantifies antibody- and magnetic nanoparticle-tagged CTCs could be useful for cancer diagnosis. In whole blood, cells were labeled with targeted antibodies and magnetic nanoparticles and then screened in a microfluidic chip-based detector that uses magnetic sensors. In 20 samples from patients with ovarian cancer, the detector quantified CTCs in 100% of patients with progressive disease, whereas the CellSearch Circulating Tumor Cell Kit quantified only 18%. Next steps include increasing the system's throughput. Johnson & Johnson's Veridex LLC unit markets CellSearch to capture and count CTCs to determine the prognosis of patients with metastatic prostate, colorectal or breast cancer.	Provisional patent filed; available for licensing	Issadore, D. <i>et al. Sci. Transl. Med.</i> ; published online July 14, 2012; doi:10.1126/scitranslmed.3003747 Contact: Ralph Weissleder, Massachusetts General Hospital, Boston, Mass. e-mail: rweissleder@mgh.harvard.edu Contact: Hakho Lee, same affiliation as above e-mail: hlee@mgh.harvard.edu
Targeted mass spectrometry via selected reaction monitoring (SRM) assays for biomarker validation	SRM assays for cancer-associated proteins could be used to detect and validate biomarkers. In human plasma and urine samples, SRM assays designed to identify more than 1,000 different cancer-associated proteins detected 184 and 408 such proteins, respectively, at concentrations as low as 10 ng/mL. In patient plasma samples, SRM assays quantified increases in cancer-associated markers in cases of ovarian cancer. Next steps include using the assays to detect and validate biomarkers.	Unpatented; licensing status not applicable	Hüttenhain, R. <i>et al. Sci. Transl. Med.</i> ; published online July 11, 2012; doi:10.1126/scitranslmed.3003989 Contact: Ruedi Aebersold, Swiss Federal Institute of Technology Zurich (ETHZ), Zurich, Switzerland e-mail: aegersold@imsb.biol.ethz.ch Contact: Ruth Hüttenhain, same affiliation as above e-mail: huettenhain@imsb.biol.ethz.ch Contact: Martin Soste, same affiliation as above e-mail: martin.soste@bc.biol.ethz.ch
Disease models			
Mouse model for amyotrophic lateral sclerosis (ALS)	Mouse studies suggest that loss of TAR DNA binding protein 43 (TDP-43; TARDBP) function could contribute to ALS. TDP-43 aggregation is associated with ALS. Mice with motor neuron-specific deletion of Tdp-43 showed accumulation of astrocytes and ubiquitinated protein in the spinal cord and had ALS-like muscle weakness and weight loss similar to another ALS model in which disease is caused by Tdp-43 aggregation. Next steps include screening for compounds that increase soluble TDP-43 levels.	Patent pending; available for licensing	Wu, L.-S. <i>et al. J. Biol. Chem.</i> ; published online June 20, 2012; doi:10.1074/jbc.M112.359000 Contact: C.-K. Shen, Academia Sinica, Taipei, Taiwan e-mail: ckshen@imb.sinica.edu.tw

This week in techniques

Approach	Summary	Licensing status	Publication and contact information
Mouse model for autism spectrum disorder (ASD) caused by mutations in <i>SH3 and multiple ankyrin repeat domains 2 (SHANK2)</i>	<p><i>Shank2</i> knockout mice could help identify potential therapeutic strategies for ASD cases caused by mutations in the gene. The knockout mice had lower hippocampal synaptic activity and greater ASD-like impairments in cognition, behavior and social functioning than wild-type controls. In the model, small molecule NMDAR agonists and metabotropic glutamate receptor subtype 5 (mGluR5; GRM5) agonists decreased ASD-like behaviors compared with vehicle. Next steps include further characterization of behavioral and physiological abnormalities caused by loss of <i>Shank2</i> function.</p> <p>Seaside Therapeutics Inc.'s GABA_B receptor agonist, STX209, and mGluR5 antagonist, STX110, are in Phase II and Phase I testing for ASD, respectively.</p> <p>SciBX 5(29); doi:10.1038/scibx.2012.769 Published online July 26, 2012</p>	Patent pending; available for licensing	<p>Won, H. <i>et al. Nature</i>; published online June 13, 2012; doi:10.1038/nature11208 Contact: Eunjoon Kim, Korea Advanced Institute of Science and Technology, Daejeon, South Korea e-mail: kime@kaist.ac.kr Contact: Min Goo Lee, Yonsei University College of Medicine, Seoul, South Korea e-mail: mlee@yuhs.ac Contact: Bong-Kiun Kaang, Seoul National University, Seoul, South Korea e-mail: kaang@snu.ac.kr</p>
Mouse model for creatine transporter deficiency (CTD)	<p>A mouse model of CTD could be useful for testing therapeutic candidates for the condition, which is caused by mutations in <i>solute carrier family 6 neurotransmitter transporter creatine member 8 (SLC6A8; CRT)</i>. Mice with brain-specific knockout of <i>Slc6a8</i> had lower creatine levels in the brain than wild-type controls and showed cognitive impairments. In the model, the membrane-permeating analog of creatine, called cyclocreatine, decreased these symptoms compared with creatine or placebo. Next steps include conducting dose-ranging studies and testing the effect of cyclocreatine on early brain development.</p> <p>Lumos Pharma Inc. has an undisclosed creatine analog in preclinical development for CTD.</p> <p>SciBX 5(29); doi:10.1038/scibx.2012.770 Published online July 26, 2012</p>	Patented; licensed to Lumos Pharma	<p>Kurosawa, Y. <i>et al. J. Clin. Invest.</i>; published online July 2, 2012; doi:10.1172/JCI59373 Contact: Joseph F. Clark, University of Cincinnati, Cincinnati, Ohio e-mail: clarkjf@gmail.com</p>
Sheep model for load-bearing long bone defects	<p>Aged sheep with defects in their long bones could be useful for evaluating bone repair and regeneration treatments. The model was used to evaluate marketed polycaprolactone-tricalcium phosphate scaffolds alone or in combination with either autologous bone marrow-derived mesenchymal stem cells (MSCs) or recombinant human bone morphogenetic protein 7 (BMP7; OP-1). In the model, scaffold plus BMP7 led to better repair of bone defects than scaffold plus MSCs and scaffold alone. Clinical trials to evaluate the scaffold plus BMP7 are under way, and the aged sheep model is being used to test a modified strategy using the scaffold plus MSCs.</p> <p>SciBX 5(29); doi:10.1038/scibx.2012.771 Published online July 26, 2012</p>	Patent application filed; available for licensing	<p>Reichert, J.C. <i>et al. Sci. Transl. Med.</i>; published online July 4, 2012; doi:10.1126/scitranslmed.3003720 Contact: Dietmar W. Huttmacher, Queensland University of Technology, Brisbane, Queensland, Australia e-mail: dietmar.huttmacher@qut.edu.au</p>
Drug delivery			
Ultrasound-stimulated microbubbles for disruption of tumor vasculature	<p>Mouse studies suggest ultrasound-stimulated disruption of tumor vasculature could be combined with radiation therapy. In a mouse xenograft model of human pancreatic cancer, i.v. microbubble injection followed by ultrasound pulses disrupted tumor vasculature. Combining the technique with gamma radiation increased cancer cell death compared with either treatment alone. Next steps include optimizing the technology's hardware and software and planning clinical trials.</p> <p>SciBX 5(29); doi:10.1038/scibx.2012.772 Published online July 26, 2012</p>	Provisional patent application filed; available for licensing	<p>Czarnota, G.J. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online July 9, 2012; doi:10.1073/pnas.1200053109 Contact: Gregory J. Czarnota, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada e-mail: gregory.czarnota@sunnybrook.ca</p>

This week in techniques

Approach	Summary	Licensing status	Publication and contact information
Drug platforms			
Topical delivery of small interfering RNA with spherical nucleic acid–nanoparticle conjugates	<p>Spherical nucleic acid–nanoparticle conjugates could be useful for topical delivery of siRNA to skin cells. The conjugates consisted of gold nanoparticles coated with covalently immobilized siRNA. In hairless mice, topical application of nanoparticles conjugated to <i>epidermal growth factor receptor (EGFR)</i>-targeting siRNA decreased <i>Egfr</i> mRNA levels compared with application of nanoparticles coated with nontargeting siRNA or nanoparticles plus free <i>EGFR</i>-targeting siRNA. In an <i>in vitro</i>, 3D model of human skin, nanoparticles coated with <i>EGFR</i>-targeting siRNA lowered cellular <i>EGFR</i> mRNA levels compared with nanoparticles coated with nontargeting siRNA. Next steps could include evaluating the efficacy of the siRNA-conjugated nanoparticles in various skin conditions including skin cancer.</p> <p>Study author Chad Mirkin cofounded AuraSense Therapeutics LLC to develop and commercialize therapies that use spherical nucleic acid constructs.</p> <p>SciBX 5(29); doi:10.1038/scibx.2012.773 Published online July 26, 2012</p>	Patented; licensed to AuraSense Therapeutics	<p>Zheng, D. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online July 6, 2012; doi:10.1073/pnas.1118425109</p> <p>Contact: Amy S. Paller, Northwestern University, Chicago, Ill. e-mail: apaller@northwestern.edu</p> <p>Contact: Chad A. Mirkin, same affiliation as above e-mail: chadnano@northwestern.edu</p>

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