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By Tracey Baas, Senior Editor

A **Harvard Medical School** team has identified a T cell-based immune response in the skin that does a better job of protecting mice from some viral infections than circulating T cells.¹ **Trem Rx Inc.** is using associated proprietary technology as the basis of a vaccine platform against infectious diseases and cancer.

"Conventional vaccines typically focus on the B cell arm of the immune system to create disease-fighting antibodies. The Trem Rx platform works to engage the T cell arm of the immune system through the generation of a newly discovered subpopulation of immune cells—the T_{RM} cells—that reside in the skin, lung, gut and other epithelial tissues," said Thomas Kupper, professor of dermatology at Harvard Medical School. Kupper is also chair of dermatology at **Brigham and Women's Hospital** and the **Dana-Farber Cancer Institute**, and he is the scientific founder of Trem Rx.

Memory T cells reside in either blood or lymphoid tissue, where they are called T central memory (T_{CM}) cells, or in epithelial tissues outside the blood and/or lymph compartment, where they are called T resident effector memory (T_{RM}) cells. An open question had been the relative contributions of T_{CM} and T_{RM} cells to protecting the host from invading pathogens.

A team led by Kupper thus set out to determine whether T_{CM} cells or T_{RM} cells provided superior protection against infection.

In mice, a vaccinia virus (VACV) infection delivered by scarification led to the production of both CD8⁺ T_{CM} and CD8⁺ T_{RM} cells that produced proinflammatory cytokines and persisted throughout the skin for at least six months postinfection.

Mice with both CD8⁺ skin T_{RM} and CD8⁺ T_{CM} cells cleared a subsequent VACV reinfection 10,000-fold more effectively than naïve mice. Similar protection was seen in mice with CD8⁺ skin T_{RM} and CD8⁺ T_{CM} cells treated with a compound that blocks movement of T_{CM} cells from lymph nodes to blood, indicating the T_{RM} cells were the primary mode of defense.

Finally, the team used a parabiotic mouse model, consisting of a previously VACV-infected mouse surgically connected to a never-infected naïve mouse, to create animals that had only CD8⁺ T_{CM} cells. While the mice were connected, CD8⁺ T_{CM} cells from the infected mouse were transferred through the shared bloodstream to the naïve mouse.

Following separation, the naïve mouse now containing CD8⁺ T_{CM} cells but not CD8⁺ skin T_{RM} cells cleared the virus only 30-fold more effectively than completely naïve mice.

Together, the findings suggest CD8⁺ T_{RM} cells provide superior protection over circulatory CD8⁺ T_{CM} cells, and a vaccine that boosts



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production of CD8⁺ skin T_{RM} cells could lead to long-lasting protective immunity against skin infections.

Results were published in *Nature*.

The new findings support work reported in *Nature Medicine* by Kupper's laboratory in 2010 that showed VACV immunization by skin scarification was superior to other injection routes—subcutaneous, intradermal, intramuscular and intraperitoneal—and provided better protection from subsequent VACV skin or respiratory challenge.² The skin scarification with VACV was shown to produce T_{CM} cells in the blood and T_{RM} cells in both the skin and lung epithelia.

Because the team did not yet have the parabiotic mouse model, they used antibody-deficient mice and mice treated with the compound that blocks movement of T_{CM} cells from lymph nodes to blood to show the animals were protected against respiratory challenge. These results suggested that lung T_{RM} cells alone were sufficient for protection; however, the protection was better when both T_{RM} and T_{CM} cells were present.

Kupper's laboratory plans to use the parabiotic mouse model

to confirm which specific CD8⁺ T cells contribute to the antiviral effects in the lung.

The skin immunization strategy used in both studies also protected mice from melanoma challenge. All mice receiving immunization by skin scarification with VACV engineered to express the melanoma antigen ovalbumin (OVA) survived a challenge with melanoma cells. In contrast, the survival rate was less than 50% for animals receiving subcutaneous, intradermal, intramuscular or intraperitoneal immunization.

"The insights gained from the studies definitely open ways for future vaccine development, but to proceed we first need more basic knowledge on these protective cell populations—what does it take to induce these T_{RM} cells, how long they will be around and how far away do they distribute," said Anke Huckriede, professor of vaccinology at the **University Medical Center Groningen**.

Thus, she said, "it has to be investigated to what extent T_{RM} cells in the skin can reduce overall virus load." Huckriede also wanted to see the studies "extended to other animal models with better predictive value for the human situation than mice."

Peter Openshaw, director of the Centre for Respiratory Infection at the National Heart and Lung Institute at **Imperial College London**, thinks demonstrating the effectiveness of skin-delivered vaccines is going to be a challenge because the skin-specific vaccine platform does not generate neutralizing antibodies.

"Currently, new batches of flu vaccines are evaluated for generating a strong immune response by using surrogate markers such as serum neutralizing antibody inhibition or hemagglutination inhibition," he

"The insights gained from the studies definitely open ways for future vaccine development, but to proceed we first need more basic knowledge on these protective cell populations—what does it take to induce these T_{RM} cells, how long they will be around and how far away do they distribute."

—Anke Huckriede,
**University Medical Center
 Groningen**

said. “Although these markers may show that the vaccine satisfies the regulatory criteria, it may not actually be effective against the targeted disease. To actually test effectiveness, the evaluation process is tough, a real challenge.”

Kupper said his team has strategies to evaluate the vaccine using blood and very small skin biopsies.

Choosing the right diseases

Trem Rx’s vaccines incorporate replication-deficient viruses. The company declined to disclose its lead indications, but President Eric Stromquist said potential areas of interest include HPV, influenza and HIV.

In HPV, Gardasil from **Merck & Co. Inc.** and **Sanofi** is marketed to prevent infection. The vaccine is based on virus-like particles made up of recombinant capsid proteins of HPV types 6, 11, 16 and 18, all of which are HPV subtypes associated with cervical cancer.

Rachael Clark, assistant professor at Harvard Medical School and one of Kupper’s collaborators, was the first to carefully study T_{RM} cells in human skin, demonstrating that there were twice as many T cells in skin as in blood. She has been collaborating with groups studying other human tissues and has identified T_{RM} cells in cervical epithelial tissue,³ leading these investigators to hypothesize that boosting this population of protective T cells could help eradicate HPV-infected epithelial cells, thus reducing the risk of malignant transformation.

In the case of influenza, Stromquist said, the conventional trivalent vaccine “needs to be decided upon every year, and sometimes it is a hit or miss, depending on if the prediction of emerging influenza strains was accurate. A number of researchers are trying to create a universal influenza vaccine by producing a universal hemagglutinin antigen. Because our vaccine’s protection is independent of antibody production, you don’t have to worry about predicting the right antigen to use, and our platform could be used to either replace or to complement any antibody-inducing vaccine platform.”

In HIV, Kupper said the key would be creating a first line of defense at the epithelial layer of the reproductive mucosa. “I think that a vaccine platform that induces protective T_{RM} cells in relevant epithelial tissues, such as the reproductive mucosa, might have a better chance of preventing HIV infection than vaccine platforms optimized to induce T_{CM} cells or produce antibodies in the blood,” he said. “By the time HIV infection takes hold in the epithelial tissues, it is very difficult to eradicate.”

Trem Rx’s other areas of interest include respiratory syncytial virus (RSV), polio, tuberculosis and intracellular bacterial infections.

Although the company’s vaccines are in preclinical development, there is clinical evidence supporting the hypothesis that T_{RM} cells provide superior protection over circulatory T_{CM} cells in humans.

In January 2012, Clark and Kupper published results from a study on the immunological effects of Sanofi’s Campath alemtuzumab in 18 patients with leukemic cutaneous T cell lymphoma (CTCL), a malignancy of skin-homing TCM cells.³ Patient skin and blood samples showed the drug depleted malignant T_{CM} cells and B cells circulating

“I think we are beginning to reappraise skin scarification for delivery of vaccines. It should not be considered an archaic method from the history of medicine. Most vaccines today are injected into muscle, bypassing the innate and adaptive immune effectors of both the epidermis and dermis.”

—Thomas Kupper,
Harvard Medical School

in the blood but spared normal T_{RM} cells in the skin. Despite the complete absence of T and B cells in the blood, 17 patients did not experience any infections.

These data give further support to the idea that T_{RM} cells alone can provide immunologic protection against infection.

Leukemic CTCL is often refractory to multiple therapies. Median survival is three years, and most patients die from uncontrolled infections due to dysfunction of the immune system.

Campath is a humanized mAb against CD52 that is marketed to treat chronic lymphocytic leukemia (CLL).

Back to the future

Vaccination through skin scarification is hardly a new idea, as it is how the smallpox vaccine is delivered. The current data provide a mechanistic explanation for the clinical success of the approach.

“I think we are beginning to reappraise skin scarification for delivery of vaccines. It should not be considered an archaic method from the history of medicine,” Kupper said. “Most vaccines today are injected into muscle, bypassing the innate and adaptive immune effectors of both the epidermis and dermis.”

He added, “While scarification works, there are a number of approaches being worked on outside of our laboratory and outside of Trem Rx to deliver vaccines to skin.”

Huckriede noted that although scarification has a long track record of human use, “now there are alternative techniques to penetrate the stratum corneum. These include intradermal injection, microneedle-based delivery, tattooing, jet injectors or patches. It needs to be evaluated which of these techniques would work best for the induction of skin-resident T cells, as this might depend on the exact type of antigen-presenting cells targeted.”

The results in the *Nature* and *Nature Medicine* papers are patented by Kupper and colleagues, and Brigham and Women’s Hospital. The IP has been assigned to Trem Rx.

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PARP target practice

By Joanne Kotz, Senior Editor

Swedish researchers have used an *in vitro* screen to evaluate the target selectivity of a panel of small molecule poly(ADP-ribose) polymerase inhibitors.¹ The findings could eventually lead to next-generation compounds with a better therapeutic index than those now in the clinic for cancer.

The 17 human poly(ADP-ribose) polymerase (PARP) enzymes attach poly-ADP-ribose or mono-ADP-ribose to target proteins. The best-characterized PARP family members are the DNA repair enzymes PARP1 and PARP2. The tankyrases, TNKS1 and TNKS2, are members of the PARP family that are involved in the DNA damage response as well as regulation of wingless-type MMTV integration site (WNT) signaling. The function of many of the remaining PARP enzymes is poorly understood.

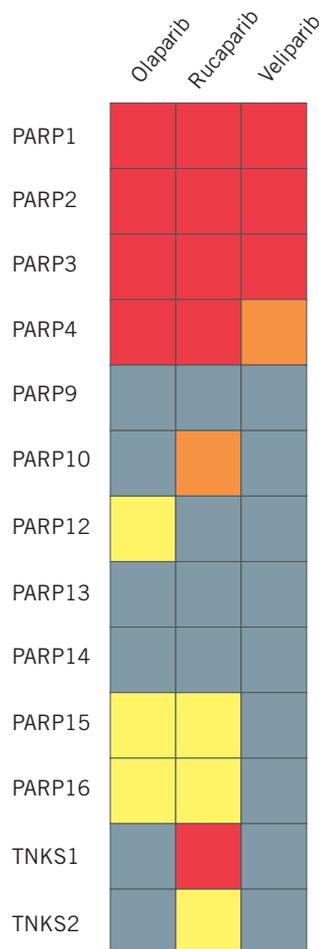
Clinical interest in PARPs heated up in 2005 when two back-to-back papers in *Nature* showed that PARP inhibitors selectively killed

Figure 1. PARP inhibitors in profile. A Swedish research team has profiled the selectivity of a panel of poly(ADP-ribose) polymerase (PARP) inhibitors, including three inhibitors in Phase II trials for cancer—olaparib (AZD2281) from **AstraZeneca plc**, veliparib (ABT-888) from **Abbott Laboratories** and rucaparib (CO-338) from **Clovis Oncology Inc.**, **Pfizer Inc.** and **Cancer Research UK**.

The team used an *in vitro* assay that quantified binding, a surrogate measure for inhibitor potency, of 185 PARP inhibitors to 13 of the 17 human PARP enzymes, including the tankyrases, TNKS1 and TNKS2.

In the heat map, red indicates strong binding, orange signifies intermediate binding and yellow represents weak binding. These binding measurement ranges correspond to IC_{50} values of approximately low to mid nanomolar, mid to high nanomolar and high nanomolar to low micromolar, respectively. Gray indicates no detectable binding.

Of the three clinical PARP inhibitors profiled, veliparib was the most selective, binding strongly to PARP1–3, and rucaparib the most promiscuous, binding detectably to nine PARP domains.



cells and tumors with deficiencies in the DNA repair enzymes *breast cancer 1 early onset (BRCA1)* or *BRCA2*.^{2,3} Loss-of-function genetic mutations in *BRCA1* or *BRCA2* occur in multiple cancers, notably breast and ovarian.

At least three PARP inhibitors are in Phase II testing in various cancers—olaparib (AZD2281) from **AstraZeneca plc**, veliparib (ABT-888) from **Abbott Laboratories** and rucaparib (CO-338) from **Clovis Oncology Inc.**, **Pfizer Inc.** and **Cancer Research UK**.

In December 2011, AstraZeneca discontinued development of olaparib for serous ovarian cancer based on an interim analysis of Phase II data suggesting the molecule would not lead to a sufficient overall survival benefit.

Although many first-generation PARP inhibitors were developed against PARP1, the specificity of the inhibitors for PARP1 over other PARP family members has not been systematically studied. A team led by Herwig Schüler thus set out to profile the selectivity of these PARP inhibitors.

At the time of the study, Schüler was a principal investigator at the **Structural Genomics Consortium** site in Stockholm. That site has since been shuttered, and Schüler is now a principal investigator in the Department of Medical Biochemistry and Biophysics at the **Karolinska Institute**.

First, the Swedish team expressed and purified the catalytic domains from 13 of the 17 human PARP enzymes. The team then established an *in vitro* assay for measuring ligand binding to the domains, a surrogate measure for inhibitor potency that was used because enzymatic assays are not available for all of the PARP enzymes.

Using this assay, the researchers quantified the binding of 185 known and potential PARP inhibitors to the 13 PARP domains. The vast majority of the molecules were promiscuous, binding to multiple PARP family members.

Of the three clinical PARP inhibitors profiled, veliparib was the most selective, binding strongly to PARP1–3 and detectably to two additional PARP family members. Olaparib bound strongly to PARP1–4 and weakly to three additional PARP enzymes. Rucaparib was the most promiscuous—binding strongly to PARP1–4 and TNKS1, as well as more weakly to four additional PARP family members including TNKS2 (see Figure 1, “PARP inhibitors in profile”).

Results were published in *Nature Biotechnology*.

The selectivity difference

The *Nature Biotechnology* paper is “an important first systematic profiling study, and the selectivity of PARP inhibitors for the different family members could be extremely important in terms of their use as chemical tools and drugs,” said Paul Workman, deputy CEO and director of the Cancer Research UK Cancer Therapeutics Unit at **The Institute of Cancer Research**.

“We don’t yet know if a pan-PARP inhibitor versus a more selective PARP inhibitor is better therapeutically and in which contexts inhibitors with different selectivity profiles may have value.”

—Paul Workman,
The Institute of Cancer Research

“We don’t yet know if a pan-PARP inhibitor versus a more selective PARP inhibitor is better therapeutically and in which contexts inhibitors with different selectivity profiles may have value. For example, hitting

“These are interesting data but binding data only. We obviously will be pursuing this to further characterize our molecule [rucaparib] to see if it is truly inhibitory for TNKS1 and TNKS2 and whether that imparts additional utility to the molecule as a cancer therapeutic.”

—Andrew Allen,
Clovis Oncology Inc.

the tankyrases as well as PARP 1–4 could have therapeutic significance but could also lead to side effects,” Workman added.

Indeed, a key question now for Clovis is whether the broader selectivity profile of its compound will translate into a better therapeutic index than the more selective veliparib and olaparib.

“These are interesting data but binding data

only. We obviously will be pursuing this to further characterize our molecule [rucaparib] to see if it is truly inhibitory for TNKS1 and TNKS2 and whether that imparts additional utility to the molecule as a cancer therapeutic,” said Andrew Allen, EVP of clinical and preclinical development and CMO of Clovis.

Allen cited results published by **Novartis AG** in *Nature* in 2009 that inhibiting TNKS1 and TNKS2 antagonizes WNT signaling.⁴ He added that WNT signaling is a “validated target, most classically in colorectal cancer but also in additional cancers including breast and melanoma.”

Thus, Allen said that Clovis will now be looking preclinically to see if inhibiting tankyrases in combination with inhibiting PARP1 and PARP2 could be synergistic in breast cancer patients with *BRCA* mutations or in a different cancer patient population. Alternatively, tankyrase inhibition could open up new cancer indications for the molecule.

The company will investigate these possibilities by comparing rucaparib to clinical-stage PARP inhibitors with distinct binding profiles, particularly AstraZeneca’s olaparib, in *in vitro* enzymatic assays, cell models and animal models, Allen added.

Alternatively, rucaparib could have greater toxicity than more selective PARP inhibitors. “We don’t know enough to know whether tankyrase inhibition could be a liability. We haven’t seen anything obvious, but whether we will see a tox profile that looks subtly different from other PARP inhibitors remains to be seen,” said Allen.

Getting particular

Next up for the Swedish team will be developing selective inhibitors as tools to clarify the function and therapeutic potential of particular PARP enzymes, said Schüler.

Schüler said the team is “trying to not overlap too much with what we believe will be targeted by pharmaceutical companies,” which he said would likely be PARP1–4 and the tankyrases.

Instead, the team is developing inhibitors against three of the other PARP family members. Although Schüler did not disclose which PARP enzymes are being targeted, potential chemical starting points for developing selective inhibitors against PARP10, PARP14 and PARP16 are discussed in the paper.

The results reported in *Nature Biotechnology* are not patented.

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Sugar free

By Lev Osherovich, Senior Writer

An international team has indirect genetic evidence that inhibiting the C isoform of fructokinase can block weight gain and insulin dysregulation in a mouse model of metabolic syndrome. The challenge now is to selectively inhibit the disease-associated form of the enzyme without compromising systemic sugar metabolism.¹

Fructokinase (ketohexokinase; KHK) catalyzes the first step in fructolysis, a sequence of enzymatic steps that breaks down dietary fructose into the smaller sugars that drive cellular metabolism. The C isoform of fructokinase is the predominant form of the enzyme in the liver, kidney and intestine, whereas the fructokinase A splice variant is expressed throughout the body.

There has been little drug development focused on blocking early steps in sugar metabolism to treat metabolic disease, mostly because it was unclear which enzyme was the best target.

“It now looks to us that inhibiting fructokinase C might be an ideal way to block sugar-associated metabolic syndrome,” said team leader Richard Johnson, professor of medicine, renal diseases and hypertension at the **University of Colorado Denver**.

High-fructose diet

Johnson’s team examined the effect of a high-fructose diet on metabolic disease markers in mice lacking both the A and C isoforms and mice lacking only the A isoform. For technical reasons, it was not possible to engineer mice lacking only the C isoform.

Mice fed a high-fructose diet and lacking both isoforms were unable to metabolize excess fructose and excreted the sugar in their urine. Mice without the A isoform absorbed fructose as effectively as wild-type mice, indicating the C isoform alone could drive fructose metabolism.

After 25 weeks on a high-fructose diet, mice lacking both isoforms of fructokinase gained less excess fat and weight and had lower blood glucose and insulin levels than wild-type mice.

The team next looked at the effect of eliminating peripheral fructokinase A. Fructokinase A knockouts had higher levels of intrahepatic fructose and fructose degradation products than wild-type controls, indicating that the liver version of the enzyme was doing double duty.

Indeed, the absence of peripheral fructokinase function led to higher fructose utilization in the liver and thus accelerated metabolic disease. Fructose-fed fructokinase A knockouts put on more weight and had greater glucose and insulin levels than wild-type controls.

“Fructokinase A was thought to be kind of an orphan enzyme without a role in fructose metabolism,” said Johnson. “There’s probably more fructokinase A in the body than fructokinase C, but fructokinase A metabolizes fructose at a slow rate. When you knock out fructokinase A, the fructose builds up and more is delivered to the liver, where it is acted upon by fructokinase C, thus leading to more fructose utilization and worse metabolic syndrome.”

“It now looks to us that inhibiting fructokinase C might be an ideal way to block sugar-associated metabolic syndrome.”

—Richard Johnson,
University of Colorado Denver

Johnson has filed a patent on targeting fructokinase C to treat metabolic disease and already has conducted screens for isoform-selective fructokinase inhibitors. He hopes to start a company based on the resulting compounds. He said an alternative would be to out-license the patent.

Fruit brute

“Understanding the implications of the metabolic problems driven by fructose overconsumption is really worthwhile,” said Thomas Hughes, president and CEO of **Zafgen Inc.** “This study suggests that inhibiting the C isozyme could overcome some of the problems associated with fructose—worsening of insulin resistance, dyslipidemia and poor utilization of fat.”

Zafgen’s beloranib (formerly ZGN-440) is an inhibitor of methionine aminopeptidase 2 (MetAP2) that is expected to enter Phase II testing for obesity this year. Hughes said inhibiting MetAP2 affects a signaling pathway that regulates energy downstream of fructokinase.

What’s missing, said Hughes, is a direct demonstration that inhibiting fructokinase C

alone can reduce fructose metabolism. In the absence of C isoform-specific knockout mice, the best way to do such an experiment may be with a small molecule inhibitor that is selective for the C isoform.

Hughes also said the high levels of plasma fructose found in mice lacking both forms of fructokinase may pose a potential safety problem. He said high blood sugar levels “raise a concern about the formation of advanced glycation end products,” which are sugar-protein adducts that compromise renal function and contribute to tissue damage in type 2 diabetes.

“Fructose is a much better glyating agent than glucose, so if you increase the levels of fructose you would expect to increase levels of advanced glycation end products, which are bad in diabetes,” said Hughes.

It’s unclear how selectively blocking fructokinase C would affect formation of such advanced glycation end products (AGEs). For now, Hughes suggested the team monitor AGE levels in mice lacking both forms of the enzyme and watch for signs of diabetes-like tissue damage.

Johnson countered that “people who lack fructokinase due to a hereditary condition live healthy lives, suggesting that this is a safe target.”

He also noted that selectively inhibiting fructokinase C, while leaving fructokinase A intact, should prevent excess fructose accumulation in the blood.

Charles Hart, VP of biology at **Threshold Pharmaceuticals Inc.**, said it would be interesting to test whether patients with metabolic disease have variations in fructokinase genes or enzyme activity that correlate with disease severity.

“The results lead to ideas around a diagnostic test that could identify people more likely to be at risk due to the genetic or epigenetic states of their fructokinase genotypes,” said Hart.

Threshold dropped development of 2-deoxyglucose (2-DG), an inhibitor of glycolysis for solid tumors, in 2011 because of strategic reasons and lack of “sufficiently dramatic clinical outcomes,” said Hart.

“The results lead to ideas around a diagnostic test that could identify people more likely to be at risk due to the genetic or epigenetic states of their fructokinase genotypes.”

—Charles Hart,
Threshold Pharmaceuticals Inc.

whether its compounds could discriminate between the two isoforms of fructokinase.

Also last year, **AstraZeneca plc** discontinued development of a trio of compounds that activated glucokinase (GCK; GK), which is an enzyme related to fructokinase that initiates the entry of glucose into glycolysis.

Last year, **Johnson & Johnson** reported that it had compounds that inhibited fructokinase.² However, a company spokesperson said J&J discontinued preclinical development of those molecules for undisclosed reasons. J&J did not disclose

AZD1656, the most advanced of these compounds, was in Phase II testing for diabetes and obesity.

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COMPANIES AND INSTITUTIONS MENTIONED

AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
Threshold Pharmaceuticals Inc. (NASDAQ:THLD), South San Francisco, Calif.
University of Colorado Denver, Aurora, Colo.
Zafgen Inc., Cambridge, Mass.

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IMI's leadoff hitter

By Kai-Jye Lou, Staff Writer

Europe's **Innovative Medicines Initiative** has recruited 7 pharmas to each contribute up to 50,000 compounds from their proprietary libraries and is pooling the molecules into a public-private partnership called the European Lead Factory. The pharmas will have access to an expanded chemical space, and academic members of the factory will be able to run experiments with molecules that are much higher in quality than what is typically found in academia.

The five-year public-private partnership will have a €169 million (\$222.2 million) budget to help build a high throughput screening center and to assemble the library. IMI hopes to designate the site of the screening center by year end and to have it up and running by early 2013.

Under the partnership, participating member companies of the **European Federation of Pharmaceutical Industries and Associations** (EFPIA) will contribute up to 50,000 compounds each to populate the initial library with at least 300,000 molecules—called the Joint European Compound Collection.

EFPIA represents the pharmaceutical industry in Europe and was jointly launched by the IMI with the European Commission in 2008.

Once fully operational, the screening center is expected to support up to 24 high throughput screening projects from its non-EFPIA members, with up to 500,000 compounds per screen. The center also will provide its EFPIA members with full copies of the compound library for up to 24 additional screening projects. These projects will be run by the EFPIA members themselves.

The overarching goal of the European Lead Factory is to provide both pharma and academics with access to the tools and facilities needed to generate high-quality hits for drug R&D.

“Ideally, what we really want to see 2–3 years into the partnership are projects that are identifying lead molecules that could be taken up and developed into actual drugs,” said Hugh Laverty, senior scientific project manager of IMI.

Opening up pharma libraries

Laverty said pharmas are becoming increasingly reliant on external sources such as research institutes, universities and smaller companies for early stage drug discovery and target research. The problem, he said, is that those sources often lack the resources, including suitable compound collections, to generate sufficient numbers of high-quality lead structures needed for such research. As a result, their productivity is low relative to internal pharma R&D efforts.

“In addition, the generation of tool compounds for basic science research may cut back on selected properties needed for drug development, including, for example, aspects related to pharmacokinetics and patentability,” Laverty told *SciBX*. “Thus, despite delivering valuable mechanistic insights into biological function, their direct impact on early pharma research efforts remains limited. The

Lead Factory is an attempt to bridge this gap translating academia's cutting-edge science to quality hit compounds with pharmaceutical potential.”

He said the European Lead Factory will address this deficiency in two ways. First, the partnership will provide its members with access to a library that should be far more comprehensive than those currently available to the individual parties.

“The public will get access to compounds in high-quality chemical libraries, and the pharmas themselves will get access to the chemical libraries of other pharmas as well as compounds generated through the partnership,” said Laverty. “This partnership will help us move past the model where one company only screens its own library for compounds that could be useful in the few select disease areas that it works in.”

Second, the partnership will provide its public members with a high throughput screening facility and the necessary resources to screen large compound libraries.

Given the proprietary nature of the compound libraries being provided by EFPIA members, all screening projects under the partnership will be carried out in a blinded fashion in which structural information on library compounds is withheld. Only members of the screening center itself will have access to both bioactivity and chemical structural information for all the compounds in the Joint European Compound Collection.

For each screening project, including those carried out by EFPIA members themselves, the screening center will generate a qualified hit list of up to 50 compounds. Structural information for compounds in the qualified hit list will be unblinded and released only to the owners of the project.

The library copies that EFPIA members receive from the screening center will be blinded and will not include any structural data on the compounds. Thus, EFPIA members will need to submit the results from their in-house screening projects back to the center, which will then provide the member with a list of up to 50 qualified hits and the associated structural information.

Laverty said rights to existing IP, such as those that cover molecules in the compound library, will be retained by their respective owners.

Rights to new IP generated from projects carried out under the European Lead Factory partnership will be negotiated by the involved parties. Laverty said IMI has a guidance for IP-related issues in place.

Call for proposals

Under the European Lead Factory partnership, EFPIA members will collaborate on projects with public and private entities that are eligible for IMI funding, collectively referred to as the Applicant Consortia. This group will include universities, small- to medium-sized enterprises, patient organizations and regulatory agencies.

The partnership's EFPIA members are **AstraZeneca plc**, **Bayer AG's** Bayer HealthCare unit, **Johnson & Johnson's** Janssen Pharmaceuticals Inc. unit, **H. Lundbeck A/S**, **Merck KGaA's** Merck-Serono S.A. unit, **Sanofi** and **UCB Group**.

Laverty said the call for proposals was just issued and the Applicant Consortia are in the process of being formed.

“Ideally, what we really want to see 2–3 years into the partnership are projects that are identifying lead molecules that could be taken up and developed into actual drugs.”

—Hugh Laverty,
Innovative Medicines Initiative

The call for proposals will occur in two stages in which members of the European Lead Factory's Applicant Consortia first submit an Expression of Interest that will be reviewed and ranked by an independent group of experts. Applicants with the highest-ranked proposals will receive an invitation to develop a full project proposal with the EFPIA Consortium, which will then be submitted for final review by an independent group. Final project proposals that receive a favorable review will receive IMI funding.

For members of the Applicant Consortia, up to 75% of the costs associated with research and technological development activities, and up to 100% of the direct costs associated with other activities such as management and training, will be eligible for IMI funding. There is a separate reimbursement schedule to cover indirect costs incurred by members of the Applicant Consortia, such as overhead. The EFPIA members themselves are not eligible for IMI funding and will be responsible for funding their own activities and projects carried out under the partnership.

Under the five-year partnership, the European Commission's 7th Framework Programme for Research will contribute up to €80 million (\$105.2 million), with €40 million (\$52.6 million) each allocated for projects related to the screening center and compound library.

The remaining €89 million (\$117 million) of the €169 million

“The public will get access to compounds in high-quality chemical libraries, and the pharma themselves will get access to the chemical libraries of other pharma as well as compounds generated through the partnership.”

—Hugh Laverty,
Innovative Medicines Initiative

(\$222.2 million) budget will primarily come in the form of in-kind contributions from EFPIA members. These contributions include the 300,000 compounds that the EFPIA members will donate to the Joint European Compound Collection, which are valued at €60 million (\$78.9 million) assuming an average cost of €200 (\$262.94) per donated compound. The scientific, technical and legal expertise EFPIA members provide to collaborators is valued at €24 million (\$31.6 million). Direct cash contributions from EFPIA members are expected to be about €5

million (\$6.6 million).

Lou, K.-J. *SciBX* 5(13); doi:10.1038/scibx.2012.325

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COMPANIES AND INSTITUTIONS MENTIONED

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

Bayer AG (Xetra:BAY), Leverkusen, Germany

European Federation of Pharmaceutical Industries and Associations, Brussels, Belgium

Innovative Medicines Initiative, Brussels, Belgium

H. Lundbeck A/S (CSE:LUN), Copenhagen, Denmark

Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.

Merck KGaA (Xetra:MRK), Darmstadt, Germany

Sanofi (Euronext:SAN; NYSE:SNY), Paris, France

UCB Group (Euronext:UCB), Brussels, Belgium

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				
Autoimmune disease	Protein tyrosine phosphatase non-receptor type 22 (PTPN22; LYP)	<i>In vitro</i> studies identified a LYP inhibitor that could help treat autoimmune diseases. Certain autoimmune diseases involve a mutation in LYP that prevents the protein from binding to c-src tyrosine kinase (CSK), thus resulting in greater CSK-mediated inhibition of T cell receptor (TCR) signaling. In T cells, the LYP inhibitor increased TCR signaling compared with vehicle control. Next steps include improving the inhibitor's potency, efficacy and selectivity, as well as testing the compound in animal models of autoimmunity.	Findings unpatented; unavailable for licensing	Vang, T. <i>et al. Nat. Chem. Biol.</i> ; published online March 18, 2012; doi:10.1038/nchembio.916 Contact: Lutz Tautz, Sanford-Burnham Medical Research Institute, La Jolla, Calif. e-mail: tautz@burnham.org
SciBX 5(13); doi:10.1038/scibx.2012.326 Published online March 29, 2012				
Cancer				
Acute lymphoblastic leukemia (ALL)	Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)	Mouse studies suggest TRAIL could eliminate cancer stem cells to help treat ALL. In mice, transplantation of human ALL cells that had been cultured with TRAIL delayed leukemia engraftment and death, and led to fewer leukemia stem cells and lower leukemia burden than transplantation of cells cultured with vehicle control. In mice with human ALL xenografts, TRAIL extended survival and prevented leukemia in three of five mice. Next steps include testing TRAIL in patients with ALL. RG7425, an anti-TRAIL mAb from Roche, is in Phase II testing to treat various solid and blood cancers.	Patent and licensing status unknown	Castro Alves, C. <i>et al. Blood</i> ; published online March 9, 2012; doi:10.1182/blood-2011-08-370114 Contact: Irmela Jeremias, German Research Center for Environmental Health, Munich, Germany e-mail: irmela.jeremias@helmholtz-muenchen.de
SciBX 5(13); doi:10.1038/scibx.2012.327 Published online March 29, 2012				
Brain cancer	VEGF receptor 2 (KDR/Flk-1; VEGFR-2)	<i>In vitro</i> and mouse studies suggest inhibiting VEGFR-2 could help treat glioblastoma multiforme (GBM). In glioma stem cells, cell surface expression of VEGFR-2 was greater than that in nonstem glioma cells. In cultured GBM cells, small hairpin RNA against VEGFR-2 increased apoptosis compared with control shRNA. In xenograft mice implanted with GBM cells, a VEGFR-2 kinase inhibitor decreased tumor formation and increased survival compared with vehicle or the anti-VEGF antibody Avastin bevacizumab. Next steps could include testing VEGFR-2-specific inhibition in additional GBM disease models. Roche's Genentech Inc. unit and Chugai Pharmaceutical Co. Ltd. market Avastin to treat several cancers including GBM.	Patent and licensing status unavailable	Hamerlik, P. <i>et al. J. Exp. Med.</i> ; published online March 5, 2012; doi:10.1084/jem.20111424 Contact: Jiri Bartek, Danish Cancer Society Research Center and Centre for Genotoxic Stress Research, Copenhagen, Denmark e-mail: jb@cancer.dk Contact: Jeremy N. Rich, Cleveland Clinic, Cleveland, Ohio e-mail: richj@ccf.org
SciBX 5(13); doi:10.1038/scibx.2012.328 Published online March 29, 2012				

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Breast cancer	Euchromatic histone-lysine N-methyltransferase 2 (EHMT2; G9A); DNA methyltransferase	Cell culture and mouse studies suggest blocking both G9A histone methyltransferase activity and its interactions with DNA methyltransferases could help treat breast cancer. In a mouse xenograft model of breast cancer, small hairpin RNA-mediated knockdown of G9A lowered tumor growth and metastasis compared with G9A expression. In cultured breast cancer cells, G9A shRNA decreased a DNA methyltransferase-mediated interaction associated with epithelial-to-mesenchymal transition compared with control shRNA. Next steps include screening for small molecules that disrupt the interaction of G9A with DNA methyltransferases. SciBX 5(13); doi:10.1038/scibx.2012.329 Published online March 29, 2012	Unpatented; licensing status not applicable	Dong, C. <i>et al. J. Clin. Invest.</i> ; published online March 12, 2012; doi:10.1172/JCI57349 Contact: Binhua P. Zhou, University of Kentucky College of Medicine, Lexington, Ky. e-mail: peter.zhou@uky.edu
Cancer	Cytoplasmic dynein	<i>In vitro</i> and cell culture studies identified small molecule inhibitors of cytoplasmic dynein that could help treat cancer. Dynein is a microtubule motor protein required for proper function of the primary cilium, a key regulator of hedgehog signaling. In cell culture, the small molecule inhibitors, named ciliobrevins, disrupted the localization of hedgehog components to the primary cilium and inhibited hedgehog signaling. <i>In vitro</i> , the compounds inhibited dynein ATPase activity, whereas an inactive analog did not. Next steps include developing more potent inhibitors that are selective for different isoforms of cytoplasmic dynein. SciBX 5(13); doi:10.1038/scibx.2012.330 Published online March 29, 2012	Patent application filed; available for licensing	Firestone, A.J. <i>et al. Nature</i> ; published online March 18, 2012; doi:10.1038/nature10936 Contact: James K. Chen, Stanford University School of Medicine, Stanford, Calif. e-mail: jameschen@stanford.edu Contact: Tarun M. Kapoor, The Rockefeller University, New York, N.Y. e-mail: kapoor@rockefeller.edu
Cancer	Interferon- β (IFNB; IFN- β)	Mouse studies suggest a synthetic sialyl-IFNB compound could help treat cancer. In a mouse model of human Burkitt's lymphoma, a synthetic IFNB compound containing a disialyloligosaccharide decreased tumor growth compared with fibroblast-derived human IFNB or vehicle. Next steps include scaling up synthesis of the sialyl-IFNB compound and evaluating its effect in additional cancers, HCV and multiple sclerosis (MS). SciBX 5(13); doi:10.1038/scibx.2012.331 Published online March 29, 2012	Patent application filed; licensed by Otsuka Chemical Co. Ltd. Contact: Katsura Torii, Otsuka Chemical Co. Ltd., Osaka, Japan e-mail: katsura.torii@otsukac.co.jp	Sakamoto, I. <i>et al. J. Am. Chem. Soc.</i> ; published online March 10, 2012; doi:10.1021/ja2109079 Contact: Yasuhiro Kajihara, Osaka University, Toyonaka, Japan e-mail: kajihara@chem.sci.osaka-u.ac.jp
Cancer	Not applicable	<i>In vitro</i> and mouse studies identified an isomer of 2-(4-methoxyphenyl)-4-quinolinyl) 2-piperidinyl-methanol that could help treat multidrug-resistant (MDR) cancers. In MDR cancer cell lines, a stereoisomer of the antimalarial compound restored sensitivity to anticancer drugs, including paclitaxel, doxorubicin and mitoxantrone, whereas the parent compound did not. In a mouse xenograft model of MDR ovarian cancer, paclitaxel plus the isomer decreased tumor growth compared with either compound alone. Next steps could include testing the compound in additional animal models. SciBX 5(13); doi:10.1038/scibx.2012.332 Published online March 29, 2012	Patent and licensing status unavailable	Duan, Z. <i>et al. J. Med. Chem.</i> ; published online March 8, 2012; doi:10.1021/jm300117u Contact: Zhenfeng Duan, Harvard Medical School, Boston, Mass. e-mail: duanz@helix.mgh.harvard.edu

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1; RAC1)	<i>In vitro</i> studies identified a RAC1 inhibitor that could help treat metastatic cancers. In a RAC1-overexpressing metastatic breast cancer cell line, a derivative of the RAC1 inhibitor NSC3766 blocked RAC1 activity with 100-fold better potency than the parent compound and an IC ₅₀ value of 1.1 μM. In metastatic melanoma and breast cancer cell lines, 5 μM of the compound inhibited cell growth by 50% compared with vehicle. Next steps include testing the compound's efficacy in a mouse model. SciBX 5(13); doi:10.1038/scibx.2012.333 Published online March 29, 2012	Patent application filed; available for licensing	Montalvo-Ortiz, B.L. <i>et al. J. Biol. Chem.</i> ; published online March 1, 2012; doi:10.1074/jbc.M111.334524 Contact: Suranganie Dharmawardhane, University of Puerto Rico, San Juan, Puerto Rico e-mail: su.d@upr.edu Contact: Cornelis P. Vlaar, same affiliation as above e-mail: cornelis.vlaar@upr.edu
Lymphoma	Epstein-Barr nuclear antigen 3B (EBNA3B)	Mouse and patient sample studies suggest increasing EBNA3B signaling could help prevent Epstein-Barr virus (EBV)-associated lymphoma. In humanized mice, infection with an <i>Ebna3b</i> knockout EBV strain resulted in tumor formation and greater B cell expansion than infection with an <i>Ebna3b</i> -expressing strain. B cells derived from two patients with post-transplant lymphoproliferative disease had EBNA3B mutations. Next steps could include identifying a pharmacological agent that increases EBNA3B signaling and developing a diagnostic to screen patients for infection with <i>Ebna3b</i> -mutant EBV. SciBX 5(13); doi:10.1038/scibx.2012.334 Published online March 29, 2012	Patent and licensing status unavailable	White, R.E. <i>et al. J. Clin. Invest.</i> ; published online March 12, 2012; doi:10.1172/JCI58092 Contact: Martin J. Allday, Imperial College London, London, U.K. e-mail: m.allday@imperial.ac.uk
Ovarian cancer	Leukotriene B4 type 2 receptor (BLT2)	<i>In vitro</i> and mouse studies suggest inhibiting BLT2 could help reduce ovarian cancer metastasis. In invasive human ovarian cancer cell lines, BLT2 expression was greater than that in less invasive cell lines. In cultured invasive ovarian cancer cells, small interfering RNA against BLT2 decreased invasion compared with scrambled siRNA control. In a mouse model of ovarian cancer metastasis, a BLT2 inhibitor lowered metastasis compared with vehicle control. Next steps could include testing BLT2 inhibition in additional cancer models. SciBX 5(13); doi:10.1038/scibx.2012.335 Published online March 29, 2012	Patent and licensing status unavailable	Seo, J.-M. <i>et al. J. Biol. Chem.</i> ; published online March 6, 2012; doi:10.1074/jbc.M111.317131 Contact: Jae-Hong Kim, Korea University, Seoul, South Korea e-mail: jhongkim@korea.ac.kr
Cardiovascular disease				
Fibrillation; arrhythmia	Potassium channel Kv1.5 (KCNA5)	<i>In vitro</i> and <i>in vivo</i> studies suggest a new class of KCNA5 inhibitors could help treat atrial fibrillation (AF). In a KCNA5-expressing mouse cell line, a dihydropyrazolopyrimidine analog inhibited KCNA5 with a nanomolar IC ₅₀ value. In a rabbit model of AF, the compound increased the atrial effective refractory period, a measure of normal cardiac rhythm, compared with vehicle. In normal rats and dogs, the compound had good pharmacokinetics and no toxicity. Future studies could include testing the lead compound in other animal models of fibrillation and/or arrhythmia. SciBX 5(13); doi:10.1038/scibx.2012.336 Published online March 29, 2012	Patented by Bristol-Myers Squibb Co.; licensing status unavailable	Finlay, H.J. <i>et al. J. Med. Chem.</i> ; published online March 12, 2012; doi:10.1021/jm201386u Contact: Heather J. Finlay, Bristol-Myers Squibb Co., Princeton, N.J. e-mail: heather.finlay@bms.com Contact: John Lloyd, same affiliation as above e-mail: john.lloyd@bms.com

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Dermatology				
Dermatitis	Cysteinyl leukotriene receptor 2 (CYSLTR2; CysLT2); leukotriene C4 synthase (LTC4S)	<p>Mouse studies suggest inhibiting leukotriene C4 signaling through LTC4S and CysLT2 could help treat atopic dermatitis. In a mouse model of atopic dermatitis, <i>Ltc4s</i> or <i>Cyslt2</i> knockout decreased allergen-induced skin thickening and collagen deposition compared with wild-type expression. Next steps could include screening for small molecule inhibitors of LTC4S and CysLT2.</p> <p>SciBX 5(13); doi:10.1038/scibx.2012.337 Published online March 29, 2012</p>	Patent and licensing status unavailable	<p>Oyoshi, M.K. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online March 13, 2012; doi:10.1073/pnas.1203127109</p> <p>Contact: Raif S. Geha, Harvard Medical School, Boston, Mass. e-mail: raif.geha@childrens.harvard.edu</p> <p>Contact: K. Frank Austen, same affiliation as above e-mail: fausten@rics.bwh.harvard.edu</p>
Endocrine/metabolic disease				
Diabetes	Sphingosine-1-phosphate phosphatase 1 (SGPP1)	<p>Cell culture studies suggest antagonizing SGPP1 could help treat type 2 diabetes. In cultured mouse pancreatic islet cells, pharmacological blockade of sphingosine-1-phosphate production decreased glucose-induced insulin secretion compared with no blockade. In these cells, small interfering RNA against SGPP1, which dephosphorylates sphingosine-1-phosphate, increased sphingosine-1-phosphate and insulin levels compared with nontargeted control siRNA. Next steps include identifying SGPP1 inhibitors.</p> <p>SciBX 5(13); doi:10.1038/scibx.2012.338 Published online March 29, 2012</p>	Unpatented; licensing status not applicable	<p>Stanford, J.C. <i>et al. J. Biol. Chem.</i>; published online March 2, 2012; doi:10.1074/jbc.M111.268185</p> <p>Contact: Sabire Ozcan, University of Kentucky, Lexington, Ky. e-mail: sozcan@uky.edu</p>
Metabolic syndrome; obesity	Fructokinase (ketohehexokinase; KHK)	<p>Mouse studies suggest antagonizing the C isoform of fructokinase could help treat metabolic syndrome and obesity. <i>Fructokinase</i> knockout mice fed a high-fructose diet had lower fatty acid metabolite levels, epididymal fat and weight gain compared with wild-type controls fed a similar diet. Fructose-fed mice that lacked the universally expressed A isoform of <i>fructokinase</i> but still expressed the liver-, intestine- and kidney-specific C isoform had higher fatty acid metabolite levels, epididymal fat and body weight than wild-type controls. Next steps include optimizing antagonists of the C isoform of <i>fructokinase</i> and testing them in mouse models of metabolic syndrome and obesity.</p> <p>Johnson & Johnson has fructokinase antagonists in preclinical development for metabolic indications (<i>see Sugar free, page 6</i>).</p> <p>SciBX 5(13); doi:10.1038/scibx.2012.339 Published online March 29, 2012</p>	Patent pending; available for licensing	<p>Ishimoto, T. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Feb. 27, 2012; doi:10.1073/pnas.1119908109</p> <p>Contact: Richard J. Johnson, University of Colorado Denver, Aurora, Colo. e-mail: richard.johnson@ucdenver.edu</p>
Obesity	Phosphoinositide 3-kinase (PI3K); PI3K α ; PI3K δ ; PTEN (MMAC1; TEP1)	<p>Mouse studies suggest inhibiting PI3K could help treat obesity. In transgenic mice expressing about twice as much Pten (a PI3k regulator) as wild-type mice, age-associated metabolic pathologies were decreased and energy expenditure was increased. In the Pten transgenic mice or in wild-type mice treated with the PI3Kα and PI3Kδ inhibitor CNIO-PI3Ki, brown adipose tissue activity, energy expenditure and lifespan were greater than those in untreated wild-type mice. Ongoing studies include testing the effect of PI3K inhibition in obese and lean mice.</p> <p>SciBX 5(13); doi:10.1038/scibx.2012.340 Published online March 29, 2012</p>	Patent applications filed; available for licensing	<p>Ortega-Molina, A. <i>et al. Cell Metab.</i>; published online March 6, 2012; doi:10.1016/j.cmet.2012.02.001</p> <p>Contact: Manuel Serrano, Spanish National Cancer Research Center, Madrid, Spain e-mail: mserrano@cni.es</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Gastrointestinal disease				
Colitis	Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5; GPR49)	Cell culture and mouse studies suggest engraftment of stem cell-derived colon tissues could help treat colitis and other gastrointestinal epithelial injuries. Isolated <i>Lgr5</i> ⁺ stem cells were expanded in culture to produce a single layer of colonic tissue that could be propagated for more than 10 weeks. In a mouse model of colitis, colonic delivery of the cultured tissue resulted in long-term engraftment and repair of damaged colonic epithelia and led to higher body weights than those in ungrafted controls. Next steps include refining the culture method and conforming it to GMP standards. SciBX 5(13); doi:10.1038/scibx.2012.341 Published online March 29, 2012	Patent application filed by Tokyo Medical and Dental University; available for licensing	Yui, S. <i>et al. Nat. Med.</i> ; published online March 11, 2012; doi:10.1038/nm.2695 Contact: Mamoru Watanabe, Tokyo Medical and Dental University, Tokyo, Japan e-mail: mamoru.gast@tmd.ac.jp Contact: Hans Clevers, Hubrecht Institute and University Medical Center Utrecht, Utrecht, the Netherlands e-mail: h.clevers@hubrecht.eu
Musculoskeletal disease				
Osteoporosis	Not applicable	<i>In vitro</i> and mouse studies suggest betulinic acid analogs could help treat osteoporosis. An osteoclast differentiation assay identified a betulinic acid analog that inhibited the differentiation of mouse macrophage cells into mature osteoclasts with a nanomolar IC ₅₀ value and prevented expression of osteoclastogenesis markers without obvious cytotoxicity. In an ovariectomized mouse model of osteoporosis, the compound inhibited osteoclastogenesis and decreased bone loss compared with vehicle. Ongoing work includes lead optimization and identification of the molecular target of the betulinic acid analogs. SciBX 5(13); doi:10.1038/scibx.2012.342 Published online March 29, 2012	Patented by East China Normal University; available for licensing or partnering	Xu, J. <i>et al. J. Med. Chem.</i> ; published online March 21, 2012; doi:10.1021/jm201540h Contact: Jie Tang, East China Normal University, Shanghai, China e-mail: jtang@chem.ecnu.edu.cn Contact: Wenwei Qiu, same affiliation as above e-mail: wwqiu@chem.ecnu.edu.cn Contact: Mingyao Liu, same affiliation as above e-mail: myliu@bio.ecnu.edu.cn
Neurology				
Neuroinflammation	Cannabinoid CB ₂ receptor (CNR2)	Tissue, cell culture and mouse studies suggest agonizing CNR2 could help treat brain inflammation. Brain endothelial tissue from patients with HIV-1-associated brain inflammation and cultured human brain endothelial cells treated with proinflammatory cytokines had higher CNR2 levels than healthy and vehicle-treated controls, respectively. In a mouse model of brain inflammation, a selective CNR2 agonist decreased leukocyte adhesion to brain blood vessels and increased blood brain barrier integrity compared with vehicle. Next steps include screening and optimizing CNR2 agonists and testing them in mouse models of neurodegenerative disease, stroke and viral encephalitis. Discovery stage CNR2 agonists from Organix Inc. were used in this study. At least six other companies have CNR2 agonists in Phase I testing or earlier to treat pain, autoimmunity and inflammatory indications. SciBX 5(13); doi:10.1038/scibx.2012.343 Published online March 29, 2012	Unpatented; licensing status not applicable	Ramirez, S.H. <i>et al. J. Neurosci.</i> ; published online March 21, 2012; doi:10.1523/JNEUROSCI.4628-11.2012 Contact: Yuri Persidsky, Temple University School of Medicine, Philadelphia, Pa. e-mail: yuri.persidsky@tuhs.temple.edu

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Rett syndrome	Not applicable	<p>Mouse studies suggest bone marrow transplantation and enhancing microglial activity could both help treat Rett syndrome, a form of autism caused by a mutation in <i>methyl CpG binding protein 2</i> (<i>MECP2</i>; <i>RTT</i>). In a mouse model of <i>Mecp2</i>-mutant Rett syndrome, a bone marrow transplant from wild-type donor mice decreased disease pathology and increased survival compared with a transplant from <i>Mecp2</i>-mutant donor mice. In the same model, an inhibitor of microglia phagocytic activity blocked the therapeutic effects of the bone marrow transplant compared with no inhibitor. Next steps include identifying and testing enhancers of microglia phagocytic activity in animal models of Rett syndrome.</p> <p>SciBX 5(13); doi:10.1038/scibx.2012.344 Published online March 29, 2012</p>	Undisclosed; unavailable for licensing	<p>Derecki, N.C. <i>et al. Nature</i>; published online March 18, 2012; doi:10.1038/nature10907 Contact: Jonathan Kipnis, University of Virginia, Charlottesville, Va. e-mail: kipnis@virginia.edu</p>
Stroke	Hydrogen voltage-gated channel 1 (HVCN1)	<p>Studies in cell culture and in mice suggest antagonizing HVCN1 could help prevent brain damage caused by ischemic stroke. In a cell culture model of ischemic stroke, microglia from <i>Hvcn1</i> knockout mice had lower levels of reactive oxygen species (ROS) and less inflammatory activity than wild-type microglia. In a mouse model of ischemic stroke, <i>Hvcn1</i> knockouts had smaller infarct volumes and better neurological function than wild-type controls. Next steps include identifying selective antagonists of HVCN1.</p> <p>SciBX 5(13); doi:10.1038/scibx.2012.345 Published online March 29, 2012</p>	Patent pending; available for licensing	<p>Wu, L.-J. <i>et al. Nat. Neurosci.</i>; published online March 4, 2012; doi:10.1038/nn.3059 Contact: David E. Clapham, Harvard Medical School, Boston, Mass. e-mail: dclapham@enders.tch.harvard.edu</p>
Stroke	Prokineticin 2 (PROK2; Bv8); prokineticin receptor 2 (PROKR2; PKR2)	<p>Rat studies suggest inhibiting PROK2 signaling could help treat stroke. In a rat model of stroke, Prok2 was upregulated in the ischemic and infarct regions of the brain as early as one hour after stroke onset. Mice receiving a PROKR2 antagonist 30 minutes after stroke onset had lower infarct volume and better behavioral outcomes at day four than mice given vehicle. Ongoing work in the mouse models includes determining the optimal time period after stroke onset for administration of the PROKR2 antagonist.</p> <p>SciBX 5(13); doi:10.1038/scibx.2012.346 Published online March 29, 2012</p>	Patented by Stanford University and University of California, Irvine; available for licensing or partnering	<p>Cheng, M.Y. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online March 19, 2012; doi:10.1073/pnas.1113363109 Contact: Qun-Yong Zhou, University of California, Irvine, Calif. e-mail: qzhou@uci.edu Contact: Michelle Y. Cheng, Stanford University, Stanford, Calif. e-mail: mycheng@stanford.edu</p>
Transplantation				
Graft-versus-host disease (GvHD)	MicroRNA-155 (miR-155)	<p>Mouse studies suggest inhibiting miR-155 could help treat acute GvHD. In mice receiving an allogeneic hematopoietic stem cell transplant, a locked nucleic acid (LNA) anti-miR-155 decreased acute GvHD severity and increased survival compared with a control oligonucleotide. Next steps include conducting pharmacokinetic and pharmacodynamic studies to find the optimal dose of the miRNA as well as conducting animal toxicity studies.</p> <p>Santaris Pharma A/S' miravirsen, an LNA-modified phosphorothioate antisense oligonucleotide targeting miR-122, is in Phase II testing to treat HCV. The company has at least 11 other LNA-based oligonucleotides in Phase I testing or earlier to treat hypercholesterolemia and various cancers.</p> <p>SciBX 5(13); doi:10.1038/scibx.2012.347 Published online March 29, 2012</p>	LNA technology patented by Santaris; unavailable for licensing	<p>Ranganathan, P. <i>et al. Blood</i>; published online March 9, 2012; doi:10.1182/blood-2011-10-387522 Contact: Ramiro Garzon, The Ohio State University, Columbus, Ohio e-mail: ramiro.garzon@osumc.edu</p>

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
Chemistry			
Ring expansion approach for synthesizing libraries of macrolactones and macrolactams	A synthetic approach using ring expansion to generate lactone and lactam classes of macrocycles could provide new drug leads for targets that are difficult to modulate with small molecules. A series of bicyclic precursor molecules were synthesized in 4–5 steps, and oxidative ring expansion of these precursors generated a library of chemically diverse macrolactams and macrolactones. Next steps include preparing a larger library using the methodology and applying ring expansion routes to other classes of macrocycles. SciBX 5(13); doi:10.1038/scibx.2012.348 Published online March 29, 2012	Unpatented; licensing status not applicable	Kopp, F. <i>et al. Nat. Chem. Biol.</i> ; published online March 11, 2012; doi:10.1038/nchembio.911 Contact: Derek S. Tan, Memorial Sloan-Kettering Cancer Center, New York, N.Y. e-mail: tand@mskcc.org
Drug platforms			
Ester and esterase chemistry for tissue-specific drug activation and imaging	<i>In vitro</i> and cell culture studies suggest engineered esterases could be used to selectively activate dyes and prodrugs for tissue-specific imaging and drug delivery. A SAR study identified an α -cyclopropyl ester resistant to cleavage by human and fruit fly cell line esterases but sensitive to a porcine liver esterase. In cultured cells and brain slices treated with α -cyclopropyl-masked fluorescent probes, expression of porcine liver esterase led to ester cleavage and fluorescent activation. In cultured cells treated with an α -cyclopropyl prodrug of a small molecule inhibitor of mitosis, expression of porcine liver esterase activated the compound and caused mitotic arrest. Next steps include testing the tolerability of local delivery of ester-blocked compounds and transgenic esterase in animals. SciBX 5(13); doi:10.1038/scibx.2012.349 Published online March 29, 2012	Patent pending; available for licensing	Tian, L. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 12, 2012; doi:10.1073/pnas.1111943109 Contact: Luke D. Lavis, Howard Hughes Medical Institute Janelia Farm Research Campus, Ashburn, Va. e-mail: lavis@janelia.hhmi.org
Markers			
Truncating mutations in <i>titin</i> (<i>TTN</i>) as a marker of dilated cardiomyopathy	Screening for truncating mutations in <i>TTN</i> could help diagnose dilated cardiomyopathy, a weakening of the cardiac muscle that can lead to congestive heart failure. Genome sequencing identified truncating mutations in <i>TTN</i> in 27% of dilated cardiomyopathy patients, 1% of hypertrophic cardiomyopathy patients and 3% of healthy controls. Next steps could include determining how truncations in <i>TTN</i> lead to disease. SciBX 5(13); doi:10.1038/scibx.2012.350 Published online March 29, 2012	Patent application filed; available for licensing	Herman, D.S. <i>et al. N. Engl. J. Med.</i> ; published online Feb. 16, 2012; doi:10.1056/NEJMoa1110186 Contact: Christine E. Seidman, Harvard Medical School, Boston, Mass. e-mail: cseidman@genetics.med.harvard.edu

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