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Fat chance for cancer cachexia

By *Lauren Martz, Staff Writer*

With no drugs on the market and a pair of recent Phase III failures in cancer-associated cachexia, companies could be better off turning to the cause of the wasting disease rather than targeting its symptoms. Two independent academic teams have found evidence that browning of white fat is responsible for cachexia in patients with cancer and propose different strategies to block the process.

Last year, **GTx Inc.**'s enobosarm missed a primary endpoint of improving physical function in a Phase III trial in non-small cell lung cancer (NSCLC), although it met the co-primary endpoint of increasing lean mass. The compound—a selective androgen receptor modulator—is still in testing for cancer. In 2010, **Ark Therapeutics Group plc** discontinued development of its angiotensin-converting enzyme (ACE) inhibitor, Vitor, after the compound failed in a Phase III trial for cancer-associated cachexia. Both compounds address symptoms by reversing rather than preventing changes that cause the damage.

The new studies, from the **Spanish National Cancer Research Centre (CNIO)** and **Dana-Farber Cancer Institute**, provide the first mechanistic links between brown fat activation and cancer cachexia—an association that has been known for over 30 years—and suggest 2 ways to stop the decline.

Jan Nedergaard, a professor of molecular biosciences at **Stockholm University**, told *SciBX* that many studies have suggested that brown fat activation could play a role in cancer cachexia. But, she said, “over the years, a recurrent issue has been the missing molecular link between cancer and the brown adipose tissue.”

The CNIO-led group showed that brown fat activation is a consequence of cancer and that anti-inflammatory compounds can block the browning process and decrease cachexia severity.¹ The Dana-Farber group also found that cancer causes browning of white adipose tissue (WAT), but they went a step further to identify a specific tumor-secreted factor, parathyroid hormone-like hormone (PTH1LH; PTHRP), that stimulates the conversion and could also serve as a biomarker.² The team is pursuing an antibody against PTHRP for clinical use.

Cachexia is a wasting syndrome associated with several chronic diseases in addition to cancer. It involves decreased food intake as well as an imbalance in energy expenditure that cannot be corrected by supplementing nutrients. But in cancer, the condition also has severe consequences for treatment of the disease.

“Cachexia is not only the cause of a large percentage of cancer-associated deaths, it is also a big reason that chemotherapy has to be

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stopped and contributes to a poor outcome," said Michele Petruzzelli, a postdoctoral fellow at CNIO and lead author on the paper.

Although there are no marketed drugs, at least 12 companies have compounds in development for cancer-associated cachexia (see Table 1, "Cancer-associated cachexia products in clinical development").

According to John Beadle, CEO of **PsiOxus Therapeutics Ltd.**, the cachexia therapeutics in development fail to address the cause of the disease. "I believe that the treatments trialed to date have attempted to impact the symptoms of cachexia. Selective androgen receptor modulators, for example, attempt to reverse the loss of muscle, whilst ghrelin analogs attempt to reverse appetite loss. These are certainly signs and symptoms of cachexia, but they are not causative factors," he said. He added that therapeutics targeting the root cause of the disease could lead to improvements not only in muscle mass but also in fat mass and muscle strength.

The two new papers could put the conversion of WAT to brown fat front and center in the search for new therapies.

Unlike WAT, which stores energy as intracellular lipid droplets, brown and beige adipocytes are highly metabolically active and promote energy expenditure by breaking down lipids. White adipocytes can be converted to beige adipocytes by exposure to cold or by activation of β -adrenergic receptors and some immune-mediated pathways.^{3,4} Although brown adipocytes are not abundant in adults, beige adipocytes are interspersed throughout white tissue.

Both teams set out to explore the molecular basis of brown fat activation in cachexia that had been reported in rodent models of the disease and in some patients. The CNIO study was led by Erwin Wagner, head of CNIO's Genes, Development and Disease team. The Dana-Farber team was led by Bruce Spiegelman, a professor of cell biology and medicine at Dana-Farber and **Harvard Medical School**. Spiegelman

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Table 1. Cancer-associated cachexia products in clinical development. Although two compounds failed in Phase III trials for cancer cachexia, the landscape is still full of companies working to develop therapeutics for the cancer-associated form of the disease. At least 12 companies have therapeutics in clinical development for the indication. Because of the heterogeneity of the disease, multiple treatments may be needed to address cachexia caused by different types of cancer.

Source: BCIQ: BioCentury Online Intelligence

Company	Product	Description	Phase of development
Helsinn Healthcare S.A.; Chugai Pharmaceutical Co. Ltd. (Tokyo:4519); Especificos Stendhal S.A. de C.V.; Gruppo Angelini; Ono Pharmaceutical Co. Ltd. (Tokyo:4528); Specialised Therapeutics Australia Pty. Ltd.	Anamorelin	Small molecule ghrelin mimetic	Phase III
XBiotech Inc.	CA-18C3	Human mAb against IL-1 α	Phase III
Acacia Pharma Ltd.	APD209	Oral fixed-dose combination of progestin and the selective adrenergic receptor β_2 (ADRB2) agonist formoterol	Phase II
Aeterna Zentaris Inc. (TSX:AEZ; NASDAQ:AEZS)	AEZS-130	Oral ghrelin mimetic that acts as a growth hormone secretagogue	Phase II
Akela Pharma Inc.	GHRH analog	Peptide analog of growth hormone-releasing hormone (GHRH)	Phase II
Alder Biopharmaceuticals Inc. (NASDAQ:ALDR)	Clazakizumab	Humanized mAb against IL-6	Phase II
Novartis AG (NYSE:NVS; SIX:NOVN)	Bimagrumab	Human mAb against activin receptor type 2B (ACVR2B)	Phase II
Ohr Pharmaceutical Inc. (NASDAQ:OHRP)	OHR/AVR118	Broad-spectrum peptide nucleic acid immunomodulator	Phase II
PsiOxus Therapeutics Ltd.	MT-102	Dual-action anabolic and catabolic transforming agent with ADRB antagonist activity	Phase II
Rose Pharma A/S	GTP-200	Ghrelin-based compound	Phase II
	GTP-300	Ghrelin peptide	
Vicus Therapeutics LLC	VT-122	Fixed-dose combination of propranolol, a nonselective ADRB blocker, and the cyclooxygenase-2 (COX-2) inhibitor etodolac	Phase II
Ligand Pharmaceuticals Inc. (NASDAQ:LGND)	YK5211	Nonsteroidal selective androgen receptor modulator	Phase I

is also cofounder of **Ember Therapeutics Inc.**, which works on brown fat activation for metabolic diseases.

The uncoupling link to PTHRP

Both the CNIO and Dana-Farber studies used mouse models of cancer-associated cachexia to explore the disease mechanism. Whereas the Dana-Farber team used only the Lewis lung carcinoma (LLC) model, the CNIO team used a range of models including genetic, syngenic, chemical-induced and xenograft models of various cancer types. In all models, mice showed the loss of fat and skeletal muscle that is characteristic of cancer-associated cachexia.

Wagner's team showed that WAT browning began before skeletal muscle wasting, which gave the first clue that browning could be a cause of the wasting disease.

Both studies found that uncoupling protein 1 mitochondrial proton carrier (UCP1) was a key player in the process. UCP1 directs mitochondrial respiration toward thermogenesis and away from ATP synthesis and serves as a marker for brown fat activation. Wagner and colleagues demonstrated that *UCP1* expression was higher in WAT in the mouse models than in WAT from healthy controls. In addition, the group found *UCP1* expression in 7 of 8 samples of human adipose tissue from patients with colon cancer who have cachexia. No expression was found in adipose tissue samples from 20 patients with cancer who do not have cachexia.

In Spiegelman's study, cultured media from LLC cells induced *UCP1* expression in white adipocytes, which suggested that cancer cells secrete factors that promote adipose tissue browning. With this in

mind, the researchers looked for secreted proteins that were specifically upregulated in highly thermogenic LLC clones and identified PTHRP as a potential stimulator of WAT browning.

Next, the Spiegelman team tested a neutralizing antibody against PTHRP in the LLC model of cachexia. The antibody prevented weight loss and blocked adipose tissue and skeletal muscle wasting, and it decreased energy expenditure compared with control antibody.

Finally, the Dana-Farber group showed that PTHRP could be detected in the serum of 17 out of 47 patients with NSCLC or colon cancer. Those patients had lower lean body mass and resting energy expenditure than patients in whom PTHRP was undetectable.

The Dana-Farber study was published in *Nature*.

Spiegelman said that his team plans to develop a humanized antibody or other reagent that could neutralize human PTHRP. "I can't see any obvious disadvantage, as humans should not really have circulating levels of PTHRP. That said, you never know until it is tested in humans," he said.

His team is also looking into whether PTHRP is involved in cachexia caused by other types of cancers and other diseases including kidney and heart disease.

An inflammatory cause

Because inflammation occurs with cancer, Wagner's team used a syngenic mouse model of skin cancer to study whether inflammation was linked to cachexia and found that levels of IL-6 and other inflammatory markers were higher than those in healthy controls.

IL-6⁺ colon cancer cells implanted into mice caused tumor formation and cachexia, whereas IL-6⁻ cells caused tumors but no cachexia. Because

IL-6 is involved in proinflammatory responses, the results suggested that inflammation could contribute to the induction of cachexia.

To test that hypothesis, Wagner's group looked at the effect of an NSAID in the skin tumor model. Sulindac, a cyclooxygenase-2 (COX-2) inhibitor, decreased browning of subcutaneous WAT and reduced cachexia severity compared with vehicle.

Petruzzelli said that the team is now looking for new compounds that could inhibit the browning process based on this research.

"We found a strong anticachexic effect from NSAIDs, but chronic treatment is not indicated because of potentially serious side effects such as gastrointestinal bleeding," said Petruzzelli. "It is known, for example, that NSAIDs may help prevent intestinal cancer, but they are not used for the indication because the side effects outweigh the benefits."

He added, "We guess that patients would need very long treatment likely before cachexia manifests. Patients may take between 6 months and 5 years before developing cachexia, and at this moment there is no way to predict if and when it will develop. We need a very safe therapeutic or a biomarker to predict which patients will develop cachexia and when it will present to justify treatment with more risks."

His team is also trying to identify a disease-specific biomarker to guide treatment.

Data were published in *Cell Metabolism*.

Disease heterogeneity

Although researchers agree that both teams have presented promising new approaches to pursue in the cancer cachexia space, the key to clinical success with any strategy may be careful patient stratification.

"Cachexia is a highly heterogeneous disease. While we do know that in terms of the mechanism there is some degree of commonality between cachexia caused by different diseases, we need to separate cancer-associated cachexia, and maybe even cachexia caused by individual types of cancer, from other causes of the disease for clinical trial and drug development purposes," said Petruzzelli.

He added that the work in the *Nature* paper is particularly interesting because screening patients for *PTHRP* expression could help select patients likely to respond to a therapeutic that targets the biomarker.

The heterogeneity of cachexia in different patients with cancer could be another reason that therapeutic efficacy has been difficult to prove

in Phase III testing. Although it is not yet clear whether targeting a general mechanism such as inflammation could be effective in patients with cachexia, some researchers think it might be necessary to design

separate therapeutics for patients with cachexia who have different kinds of cancer or different factors contributing to brown fat activation.

The Dana-Farber team has already identified PTHRP as a therapeutic target that could help a subset of patients with cancer cachexia and serve as a way to identify that subset.

Spiegelman told *SciBX* that his team plans to apply the same strategy used to identify PTHRP in the lung cancer model to other cancer cachexia models to find factors involved in the browning process of other cancers.

Spiegelman's company Ember has no plans to pursue therapeutics for cachexia

based on this work. Dana-Farber has filed a patent application covering the work, and the IP is available for licensing. The work from the CNIO group is unpatented, but the team is interested in partnering to develop anticachexia therapeutics.

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—Michele Petruzzelli,
Spanish National Cancer Research Centre

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Ark Therapeutics Group plc (LSE:AKT), London, U.K.
Dana-Farber Cancer Institute, Boston, Mass.
Ember Therapeutics Inc., Boston, Mass.
GTx Inc. (NASDAQ:GTXI), Memphis, Tenn.
Harvard Medical School, Boston, Mass.
PsiOxus Therapeutics Ltd., Abingdon, U.K.
Spanish National Cancer Research Centre, Madrid, Spain
Stockholm University, Stockholm, Sweden

AstraZeneca taps the Cambridge wellspring

By Michael J. Haas, Associate Editor

AstraZeneca plc has unveiled plans for the R&D center and global headquarters it will build in Cambridge, U.K.—the next major step in the pharma’s strategy to improve pipeline productivity that it hopes will help it recover growth and regain ground in scientific leadership. The company expects that the new center will drive therapeutic innovation by allowing it to tap world-class research through partnerships with nearby institutes.

Last year, AstraZeneca announced plans to build the Cambridge headquarters—which would make it the first global pharma to establish a major research facility in the city. The new site is part of the pharma’s larger goal to concentrate its small molecule and biologics R&D in three places by 2016—with the Cambridge headquarters joining two existing sites in Gaithersburg, Md., and Mölndal, Sweden.

The move is one facet of a restructuring that AstraZeneca began to implement in 2012 that included discontinuing R&D at neuroscience sites in Sodertalje, Sweden, and Montreal, Quebec, Canada, and the elimination of about 2,200 R&D positions.

Menelas Pangalos told *SciBX*, “The way to do great science in pharma is changing. Excellent internal science needs to be married to outstanding external science and access to world-class scientists. Thus, to develop innovative medicines that are differentiated from the competition, we must be able to attract and retain great scientists internally and also work collaboratively with the very best external scientists”—both of which the Cambridge center will enable AstraZeneca to do.

Pangalos is EVP of innovative medicines and early development at AstraZeneca.

The new R&D center in Cambridge will have all of the capabilities needed to support discovery and development of small molecules, proteins and biologics in cancer as well the pharma’s programs in cardiovascular disease, diabetes, asthma, chronic obstructive pulmonary disease (COPD) and neuroscience, he said.

Pangalos said that AstraZeneca chose Cambridge as its new hub because of the city’s history of scientific excellence, a thriving life science ecosystem that includes 300 biotech and medical technology companies and its proximity to leading academic and research institutions such as the **University of Cambridge**, the **MRC Laboratory of Molecular Biology**, the **Wellcome Trust–MRC Institute of Metabolic Sciences**, the **Cancer Research UK Cambridge Institute**, **Addenbrooke’s Hospital**, the **Wellcome Trust Sanger Institute** and the **Babraham Institute**.

He also noted that Cambridge is one corner of a “golden triangle with London and Oxford that has a breadth, depth and quality of scientific innovation comparable to Boston and San Francisco.”

Although AstraZeneca was successful in attracting top-tier scientists to its Alderley Park facility near Manchester, Pangalos said, “we are seeing that it is significantly easier to attract world-class scientists to Cambridge” because the number and quality of its institutions make it a magnet for talent.

Taking the lead

Pangalos said that the new R&D center builds on existing collaborations the pharma already has in Cambridge, and “as our presence in Cambridge grows over the next few years—and we establish ourselves as a great scientific partner—I believe we will see further opportunities for both formal and informal interactions” with researchers at neighboring institutions.

In 2011, AstraZeneca and the **Medical Research Council** (MRC) partnered to repurpose 22 of the pharma’s deprioritized and discontinued compounds—a program that expanded in July to include 6 more pharma.¹

In February, AstraZeneca partnered with the Cancer Research UK Cambridge Institute to move up to 60 of the pharma’s scientists into the institute over the next 3 years.

In May, AstraZeneca and the MRC Laboratory of Molecular Biology partnered to fund a range of preclinical research projects on disease biology. AstraZeneca will contribute up to about £6 million (\$10 million) and the MRC up to about £3 million (\$5 million) over 5 years. Projects funded by the partnership will support the existing R&D activities of the two organizations but will not focus directly on drug development.

Also this year, AstraZeneca partnered with the MRC to create the AstraZeneca MRC UK Centre for Lead Discovery—which the new R&D center in Cambridge will house—to identify new targets and therapies to treat a range of diseases.

The MRC is always looking to work in innovative ways with industry and has a long-standing and productive relationship

with AstraZeneca, said Chris Watkins, director of translational research and industry at the MRC. The Lead Discovery center “addresses a key strategic priority for the MRC in enabling the discovery of new pathways involved in human disease through the use of novel small molecules and brings together the respective and complementary strengths of academia and industry,” he said.

Under the terms of the partnership, the MRC gains access to AstraZeneca’s library of 2 million compounds and state-of-the-art high throughput screening technology—“resources that are simply not available to U.K. academic researchers,” Watkins said.

Additionally, Watkins told *SciBX*, research at the Lead Discovery center will not be tied to specific disease areas but will focus on what the partners perceive as the most innovative and important scientific challenges. “We can be receptive to the best ideas coming out of the world-leading U.K. academic research base—wherever and whatever

“Some projects will align with AstraZeneca’s research strategy but others will not, and the pharma has shown openness and flexibility in agreeing that MRC can determine which projects use the company’s compound library and screening capabilities.”

**—Chris Watkins,
Medical Research Council**

they are—to accelerate discovery science toward patient benefit and economic growth.”

He added, “Some projects will align with AstraZeneca’s research strategy but others will not, and the pharma has shown openness and flexibility in agreeing that MRC can determine which projects use the company’s compound library and screening capabilities.”

However, Watkins said, AstraZeneca will have the first option to negotiate agreements on Lead Discovery projects that do align with its research interests.

Pangalos said that AstraZeneca and its partner institutions in Cambridge will jointly define the metrics for success and project milestones for each collaboration and will regularly review progress toward the pharma’s goal of achieving “our most critical

deliverable—getting new medicine to patients.”

According to Richard Burton, head of communications for innovative medicines and early development at AstraZeneca, the R&D center will cost the pharma about £330 million (\$552 million) to build and will receive no public funding. The center will house over 2,000 employees, including the majority of the 1,600 small molecule researchers who will relocate from the pharma’s Alderley Park facility and all of the biologics

researchers from its **MedImmune LLC** unit at the Granta Park facility outside Cambridge.

However, AstraZeneca is not waiting for the center to open before expanding its presence in Cambridge: 70 AstraZeneca scientists have already relocated to 3 interim facilities in the Cambridge area, including the current MedImmune site at Granta Park, and another 400 will move into the city by year end, Pangalos said.

The 2012 restructuring initiative cut 7,300 positions and was expected to result in about \$1.6 billion in annual savings by the end of 2014. Including the 2012 cuts, AstraZeneca has eliminated nearly 29,000 positions since beginning restructuring efforts in 2007.

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COMPANIES AND INSTITUTIONS MENTIONED

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Babraham Institute, Cambridge, U.K.
Cancer Research UK Cambridge Institute, Cambridge, U.K.
Medical Research Council, London, U.K.
MedImmune LLC, Gaithersburg, Md.
MRC Laboratory of Molecular Biology, Cambridge, U.K.
University of Cambridge, Cambridge, U.K.
Wellcome Trust–MRC Institute of Metabolic Sciences, Cambridge, U.K.
Wellcome Trust Sanger Institute, Cambridge, U.K.

“To develop innovative medicines that are differentiated from the competition, we must be able to attract and retain great scientists internally and also work collaboratively with the very best external scientists.”

—*Menelas Pangalos, AstraZeneca plc*



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Corneal perspectives

By Benjamin Boettner, Associate Editor

Two independent findings could increase the availability of tissue for corneal transplants. A **University of California, San Diego**–led team has created a new source of limbal stem cells—which are needed for clear vision—by expressing *PAX6* in skin epithelial cells, while a **Harvard Medical School**–led group showed that the ATP-binding cassette transporter *ABCB5* is a marker for selection of limbal stem cells with enhanced regenerative potential.^{1,2}

For the UCSD team, the therapeutic potential of the method might rest on whether it can produce a sufficiently homogeneous population of limbal stem cells for clinical use and find a clinically viable way to increase *PAX6* (*paired box 6*) expression. The Harvard team has licensed an *ABCB5* (ATP-binding cassette sub-family B (MDR/TAP) member 5)-specific antibody to two stem cell companies—**Rheacell GmbH & Co. KG** and **Ticeba GmbH**—that will develop it for clinical use. The terms of the deals were not disclosed.

Clear vision depends on a transparent layer of corneal epithelial cells that derive from limbal stem cells. Deficiency in those cells is a leading cause of blindness, and in most cases the only treatment is to replace them by transplantation.^{3,4}

However, to be successful, the transplant requires sufficient numbers of limbal stem cells capable of sparking regeneration. Often these can be obtained from healthy cells in the contralateral, unaffected eye, which provides an autologous supply. But that option does not exist when both eyes are affected.

Under healthy conditions, the corneal surface continuously repopulates from limbal stem cells produced in the limbus, a

niche-forming, narrow band of tissue surrounding the cornea. The cells migrate toward the cornea's center and differentiate into transparent, functional corneal epithelial cells. Corneas unable to supply sufficient limbal stem cells have an opaque, skin epithelium-like morphology.

Although faulty limbal stem cell generation has been known for some time to be a cause of corneal disease, how these cells differentiate and what factors control the process have not been clear.

Skin in the game

Kang Zhang and colleagues at UCSD partnered with researchers at **Sun Yat-sen University, Sichuan University** and **Guangzhou KangRui Biological Pharmaceutical Technology Co. Ltd.** to pinpoint the molecular drivers of limbal stem cell development with the long-term goal of creating a usable supply of cells for transplantation.

Zhang is a professor of ophthalmology at the **Shiley Eye Center at the University of California, San Diego.**

As a first step, the researchers cultured donor human limbal stem cells obtained postmortem and found the right conditions to produce an expanded, homogeneous population of cells. By modifying the culture conditions, they induced the cells to differentiate into corneal epithelial cells.

Next, the team compared the genomewide expression profiles of limbal stem cells, corneal stem cells and skin epithelial stem cells from donor skin biopsies. The researchers identified two proteins—wingless-type MMTV integration site family member 7a (*WNT7A*) and *PAX6*—that were expressed at significantly higher levels in limbal and corneal stem cells than in skin epithelial stem cells.

In addition, the researchers showed that in the limbal stem cells, *WNT7A* acts upstream of *PAX6* and stimulates its expression via frizzled homolog 5 (*FZD5*), a receptor for WNT proteins.

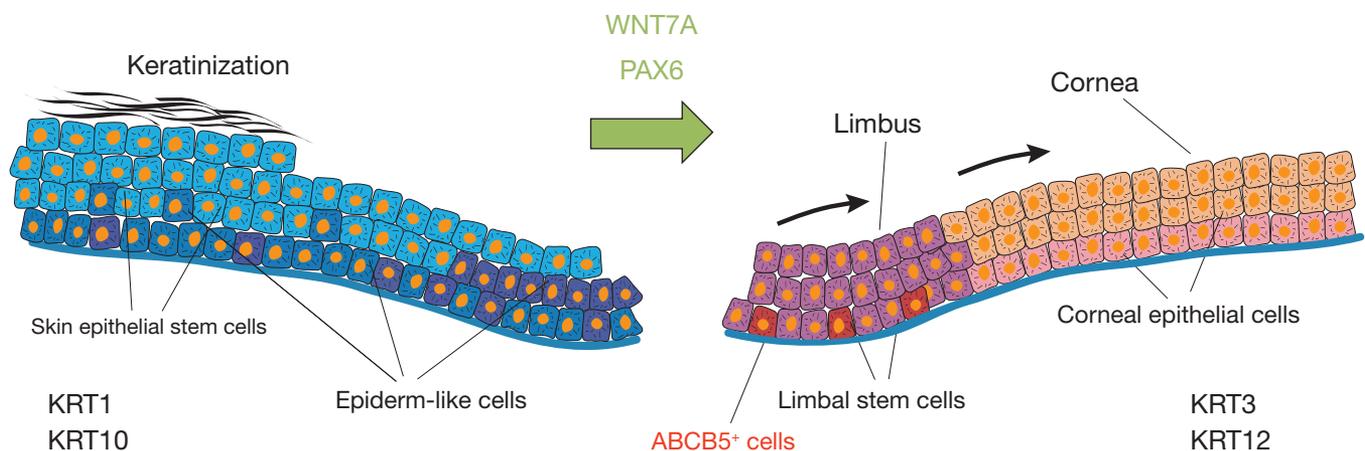


Figure 1. PAX6 drives differentiation of skin epithelial stem cells to limbal stem cells. Two new approaches that promote regenerative therapies for corneal transplants have been reported in *Nature*.^{1,2} In one study, researchers noted that *wingless-type MMTV integration site family member 7a (WNT7A)* activates *paired box 6 (PAX6)* to drive the conversion of skin epithelial stem cells to limbal stem cells that produce a healthy, clear cornea.¹ Skin epithelial cells engineered to express *PAX6* established a healthy cornea after transplantation into a rabbit model of corneal disease. In the second study, researchers identified *ATP-binding cassette sub-family B (MDR/TAP) member 5 (ABCB5)* as a marker for selection of limbal stem cells with high regenerative potential.² Loss of all three genes results in the transition of a transparent corneal epithelial morphology with keratin 3 (*KRT3*; *CK3*) and *KRT12* expression to an opaque skin epithelial morphology with *KRT1* and *KRT10* expression.

WNT7A is a secreted morphogen involved in developmental and pathogenic WNT signaling. PAX6 is a transcription factor that controls the fate and differentiation of various eye tissues.

RNAi-mediated knockdown of *WNT7A* or *PAX6* induced human limbal stem cells to transition from a corneal to a skin epithelial morphology. The *WNT7A* and *PAX6* knockdown cells also had lower expression of corneal keratin 3 (KRT3; CK3) and KRT12 and greater expression of skin epithelial KRT1 and KRT10 than wild-type limbal cells (see Figure 1, “PAX6 drives differentiation of skin epithelial stem cells to limbal stem cells”).

Conversely, overexpression of *PAX6* caused skin epithelial stem cells to revert to a limbal stem cell phenotype. The team concluded that skin epithelial cells can be reprogrammed to produce limbal stem cells by the addition of that single factor.

Finally, the team showed that rabbit skin epithelial cells engineered to express *Pax6* could re-establish a healthy cornea after transplantation into a rabbit model of limbal stem cell deficiency.

Results were published in *Nature*.

Zhang told *SciBX* that his team is now working on a *PAX6*-specific gene therapy and is testing *WNT7A* agonists as an alternative for reprogramming human skin epithelial cells to limbal stem cells.

UCSD has filed a patent covering the findings; it is available for licensing.

Conversion considerations

Because skin epithelial stem cells are far more abundant than limbal stem cells and are accessible in patients with bilateral disease, the findings suggest that skin-derived cells could provide a readily available source for limbal stem cell grafts. But it is still too soon to know how homogeneous the cultures are and what tools could reliably convert the approach to a viable system for clinical use.

Ruslan Semechkin, CSO of **International Stem Cell Corp.**, told *SciBX*, “The study provides the first direct evidence demonstrating a functional connection between *PAX6* and *WNT7A* and suggests that defects in the *WNT7A*-*PAX6* axis are likely to be responsible for metaplastic conversion of corneal cells to skin epidermal-like cells in corneal diseases in humans. It points to a new strategy for treating corneal-surface diseases.”

Michele De Luca agreed but said, “For a viable gene therapy approach with *PAX6*, it will have to be shown that skin epithelial stem cells can be reprogrammed in a safe way—for instance, under a minimal endogenous *PAX6* promoter—and that indeed a bona fide limb stem cell population is stably generated.”

According to Sophie Deng, previous studies in mice showed that skin epithelial cells can be converted to limbal stem cells when treated with corneal supernatants. “However, this is the first demonstration that human skin epithelial cells maintain the same plasticity,” she said.

In addition, she said, because *PAX6* regulates differentiation in several eye tissues, the team will need to assess the heterogeneity of the limbal stem cells produced by this system, in particular if the researchers plan to pursue gene therapy.

However, she added, “Skin epithelial stem cells will likely be a better source for transplantable limbal stem cells than induced pluripotent stem cells as they require less manipulation. Patients with bilateral corneal diseases could greatly benefit from an alternative autologous cell source.”

De Luca is a professor of biochemistry and director of the **Stefano Ferrari Center for Regenerative Medicine at the University of Modena and Reggio Emilia**. Deng is an associate professor of ophthalmology and director of the Corneal Cell Biology Laboratory at the **Jules Stein Eye Institute at the University of California, Los Angeles**.

Although *PAX6* is more likely to be used in creating a regenerative therapy, *WNT7A* could be developed directly as a therapeutic.

However, Semechkin said that option might not be straightforward. “WNT proteins are highly hydrophobic, and so far all efforts to generate fully active WNT variants have failed,” he said. But he added that *WNT7A* analogs have shown proof-of-concept in animal models of muscular dystrophy and thus could be explored for corneal disorders.

Deng suggested that small molecule screens could be performed to identify compounds that facilitate or enable conversion of skin epithelial stem cells to limbal stem cells.

But according to De Luca, *PAX6* would be a better target to focus on because *WNT7A* might have to be continuously supplied in patients with chronic corneal disease to prevent limbal stem cells from reverting to skin epithelial stem cells.

Fate Therapeutics Inc. has the biologic FT301, a *WNT7A* analog, in preclinical testing for muscular dystrophy.

Mark the limbal stem cells

In a second study, a team from Harvard Medical School and **Brigham and Women’s Hospital** wanted to find a marker to help select limbal stem cells with high regenerative potential for use in transplants.

Because stem cells in other tissues are marked by *ABC5* expression and the protein has been associated with stem cell functions, the team thought the protein could also identify limbal stem cells and might play a vital role in corneal homeostasis.

ABC5 is a member of the ATP-binding cassette superfamily of transporters that shuttles small molecules and drugs across the plasma membrane.

Using histological studies in mice, the team found that *Abcb5* is specifically expressed in the limbus and limbal stem cells and that its expression coincided with that of the corneal epithelial cell protein *Krt12* and the epithelial stem cell marker *ΔNp63*. Corneal biopsies from patients with limbal stem cell deficiencies taken postmortem

“The study provides the first direct evidence demonstrating a functional connection between *PAX6* and *WNT7A* and suggests that defects in the *WNT7A*-*PAX6* axis are likely to be responsible for metaplastic conversion of corneal cells to skin epidermal-like cells in corneal diseases in humans. It points to a new strategy for treating corneal-surface diseases.”

**—Ruslan Semechkin,
International Stem Cell Corp.**

had significantly lower levels of ABCB5 than healthy control tissue.

Knocking out *Abcb5* in mice caused apoptosis and depletion of limbal stem cells and decreased levels of corneal markers such as *Pax6* and *Krt12* compared with no alteration.

To determine if *Abcb5* could be used to select a subpopulation of limbal stem cells with high regenerative potential, the researchers developed a mAb against *Abcb5* and used it to segregate *Abcb5*⁺ from *Abcb5*⁻ limbal stem cells from mice. Segregated or unsegregated cells were then introduced into a mouse model of limbal stem cell deficiency.

Abcb5⁺ grafts yielded clear corneas within 5 weeks that had normal histology and 47% higher *Krt12* expression than corneas treated with unsegregated grafts. By contrast, *Abcb5*⁻ grafts yielded opaque corneas with no detectable *Krt12*-expressing cells.

The team was led by Natasha Frank and Markus Frank, both assistant professors at Harvard Medical School. Natasha Frank is also an associate physician at Brigham and Women's Hospital; Markus Frank is an associate physician at **Boston Children's Hospital**.

Learning the ABC of ABCB5

The identification of ABCB5 as a marker for limbal stem cells with high regenerative potential could provide another tool for improving the success of corneal transplants. The proportion of functional limbal stem cells correlates closely with the clinical success of corneal transplants.

Natasha Frank told *SciBX*, "To date, no molecular marker suitable for prospective limbal stem cell isolation has been available. Thus, our findings represent a critical first step toward isolating a molecularly defined and pure adult stem cell population. Our lab is now further dissecting the biology of human ABCB5⁺ limbal stem cells."

Natasha and Markus Frank pointed out that the predominant use of ABCB5-selected cells would be in patients with unilateral limbal stem cell deficiency who can have cells harvested from the contralateral, healthy side. But Markus Frank added that they are exploring whether the marker might also help in allogeneic transplants for patients with bilateral disease by enabling selection of cells with high regenerative potential from donors or limbal stem cell cultures.

Deng said that because the ABCB5-specific antibody labels some other cells in the mouse limbus, additional markers could help produce an even more homogeneous population. She said that her lab has shown

FZD7 maintains undifferentiated human limbal stem cells and thus could be combined with ABCB5 selection as an additional marker for limbal stem cells.⁵

OncoMed Pharmaceuticals Inc. has an anti-FZD7 antibody in Phase I trials for various cancers.

Markus Frank said, "Given the observations by [the UCSD group] and the finding that ABCB5 helps amplify a PAX6⁺ limbal stem cell population, we would also like to test whether ABCB5 selection could enhance the conversion of skin epithelial stem cells to limbal stem cells."

Brigham and Women's Hospital has filed a patent covering the findings and the anti-ABCB5 mAb. The IP has been licensed to the German stem cell companies Rheacell and Ticeba, which will develop a GMP-grade antibody for use in initial clinical trials. Markus Frank is a scientific advisor at both companies.

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OncoMed Pharmaceuticals Inc. (NASDAQ:OMED), Redwood City, Calif.
Rheacell GmbH & Co. KG, Heidelberg, Germany
Shiley Eye Center at the University of California, San Diego, La Jolla, Calif.
Sichuan University, Sichuan, China
Stefano Ferrari Center for Regenerative Medicine at the University of Modena and Reggio Emilia, Modena, Italy
Sun Yat-sen University, Guangzhou, China
Ticeba GmbH, Heidelberg, Germany
University of California, San Diego, La Jolla, Calif.

"Given the observations by [the UCSD group] and the finding that ABCB5 helps amplify a PAX6⁺ limbal stem cell population, we would also like to test whether ABCB5 selection could enhance the conversion of skin epithelial stem cells to limbal stem cells."

—Markus Frank,
Harvard Medical School

Circling back to basics

By Chris Cain, Associate Editor

Macrocycles have been a hot topic for chemists and pharmacologists alike for the promise they hold of reaching druggable targets that small molecules cannot. Although they are still much more challenging than small molecules to develop, a trio of studies might offer a way forward by proposing new design guidelines,¹ identifying protein-protein interfaces to target² and providing a new method to synthesize cyclic peptides (see Box 1, “Cyclotide synthesis”).³

The papers were published in *Nature Chemical Biology* alongside an editorial call to action for research to support macrocycle discovery and development.⁴ The proposals echoed those discussed by academic and industry leaders at *SciBX*'s 2012 Summit on Innovation in Macrocycle Drug Discovery.⁵

“Developing macrocycles is a three-component problem—what do you want to target, how do you design a macrocycle to target it and which method can you use for synthesis?” asked Adrian Whitty, an associate professor of chemistry at **Boston University**. “This whole field is at an intersection of what you would like to make, what you are able to make and what you can target. Each of those three areas has been gradually pushed forward, and in sum we are in a much better position than we were five years ago. No solution to any two of those problems can be effective without the third, and now I see all three of those areas pushing forward rapidly.”

Whitty was co-leader of one of the studies. Prior to joining Boston University and initiating this work, he was director of physical biochemistry at **Biogen Idec Inc.**

The climate for macrocycle development has changed since 2012 when, according to Joshua Kritzer, “the excitement in the field was just cresting, and now it may be starting to recede. You can now go from a peptide to a useful macrocycle in a few steps with many fewer synthetic roadblocks. But thus far it has not proven generally applicable for targeting intracellular protein-protein interactions. We know this is possible, but it's still really hard.”

Kritzer is an assistant professor of chemistry at **Tufts University** who led one of the three studies.

Spiros Liras, VP of cardiovascular, metabolic and endocrine medicinal chemistry at **Pfizer Inc.**, agreed that rational macrocycle design is still challenging.

“To be able to deliberately design permeable macrocycles takes time and commitment. Much effort is needed to break the science at a basic level and to bring this forward as a dependable modality for drug development,” he said. “The wealth of data to guide drug discovery with macrocycles and help develop guidelines is not yet available like it is in the small molecule space.”

Macrocycles meet proteins

Whitty's team embarked on a project to systematically glean as much information as possible from the limited published structural data describing macrocycle-protein binding interactions. The goal was to harness this information to guide the synthesis of compounds.

“Having come to the conclusion about four years ago that it would be fruitful to look at macrocycles as a means to disrupt intracellular protein-protein interactions, we were faced with an unanswered question as to what macrocycles we should make,” said Whitty. “So we sought to learn what kind of structural and physiochemical features were present in natural macrocycles that bind to protein targets, together with macrocycles that are approved drugs.”

Indeed, a key area highlighted by *SciBX* Summit panelists and audience members, including Liras and Whitty, was the need for a more detailed understanding of how macrocycles engage their targets.⁵

Whitty's team identified a total of 22 distinct macrocycle-protein complexes that had high-quality cocrystal structures relevant for drug discovery. The group then set out to analyze the structural features of macrocycle binding to identify shared characteristics that could inform a set of guidelines for compound development, analogous to Lipinski's rules. Those rules for small molecule drug development were published in 1995 and consolidated observations from the literature into a set of five guidelines related to drug absorption.

Whitty's team published two sets of guidelines for macrocycles. **One** focused on physiochemical properties of orally available compounds, such as size, lipophilicity and hydrogen bond donors. The **other** focused on structural features, such as ring size, the location and number of substituents and polar/nonpolar balance.¹

Scott Lokey, a professor of chemistry at the **University of California, Santa Cruz**, told *SciBX* that some of these new guidelines were not obvious. “They provide an empirical foundation for backing up—and in some cases challenging—many oft-repeated assumptions about macrocycle behavior,” he said. “One assumption that is challenged has to do with design of macrocycle drugs and the idea that the macrocycle ring is just a scaffold for the display of substituents in the proper geometry for binding the target.”

He said that in contrast, “Whitty's analysis reveals that a large number of the important contacts are made by atoms directly attached to the macrocycle, for example, hydroxyls, carbonyls and methyls. Because of their proximity to the backbone, these backbone-attached peripheral atoms also impact the overall conformation of the macrocycle, which implies that designing macrocyclic ligands will be harder than we might have thought. We'll have to go beyond the ‘macrocycle as scaffold’ paradigm and design rings that not only adopt the proper global conformation but also make productive contacts directly with the target.”

Lokey is collaborating with multiple groups, including Liras' Pfizer team, on the design of orally available macrocycles that incorporate polyketide elements, which are a feature of many natural macrocycles including rapamycin. He is also cofounder of the new macrocycle startup **Circle Pharma Inc.**

“We'll have to go beyond the ‘macrocycle as scaffold’ paradigm and design rings that not only adopt the proper global conformation but also make productive contacts directly with the target.”

**—Scott Lokey,
University of California, Santa Cruz**

Box 1. Cyclotide synthesis.

Although synthetic macrocycle chemistry has been inspired by the study of natural products such as cyclosporine, the biosynthetic pathways for many compounds remain a mystery. Now, a team led by Professor James Tam at **Nanyang Technological University** has identified a plant-produced peptide ligase that could be developed as a tool to synthesize cyclic peptides.³

Tam has long studied plants used in traditional Chinese medicine to identify bioactive compounds, and he has previously characterized members from a large and diverse family of plant-produced cyclic peptides known as cyclotides. His team sought to identify the enzyme responsible for cyclotide production in a medicinal plant, *Clitoria ternatea*.

To identify the peptide ligase that could be responsible for producing the cyclotides in *C. ternatea*, the team used liquid chromatography and fluorescent reporter constructs to isolate the activity and identify and sequence the protein responsible, which they named butelase 1.

The enzyme was purified and shown to efficiently ligate or cyclize almost any N-terminal residue to a C-terminal asparagine as long as the asparagine is encoded in an asparagine-histidine-valine sequence. The ligase also worked on non-native substrates with the tag appended.

Boston University associate professor Adrian Whitty told *SciBX*, “The impressive thing about this work is that they were able to find an enzyme that was so broad in its reactivity it requires an asparagine in one of the ligation positions but accepts almost any amino acid in the other, which allows for great freedom of design.”

PeptiDream Inc. CSO Patrick Reid said that adapting this technology for peptide-library generation is not trivial, so it is not yet clear whether the ligase will have utility for large-scale compound synthesis.

A first step will be demonstrating that a recombinant form of the enzyme can be expressed and purified.

Patent and licensing information was not available. —CC

Nick Terrett, CSO of synthetic macrocycle company **Ensemble Therapeutics Corp.**, agreed that the assessment of the binding contribution of ring atoms and substituent groups was intriguing. He was particularly interested in Whitty’s survey of the conformations in which macrocycles engage with their targets. This is a “very nice analysis of how macrocycles bind to protein surfaces, and in particular the breakdown of these interactions into edge-on, face-on and compact binding modes.”

Whitty’s analysis also showed that the region of a given macrocycle interacting with a protein was no more hydrophobic on average than the rest of the compound, suggesting that a balanced distribution of polar and nonpolar groups may be an important feature for binding protein targets.

In addition to presenting a comprehensive catalog of existing macrocycle binding interactions, Whitty’s team also used a computational solvent-mapping method, **FTMap**, to probe the sites on proteins bound by macrocycles. The team concluded that the majority of the sites would not be bound with high affinity by traditional small molecules, providing evidence that the distinct properties of macrocycles were indeed opening up new targets that otherwise could not be drugged. This work was done in collaboration with the group of Sandor Vajda, a professor of chemistry at Boston University.

“This analysis told us these target sites have binding energy hot spots, but they are spaced too far apart to be spanned by the structure of an ordinary-sized drug molecule,” said Whitty.

Patrick Reid, cofounder and CSO of cyclic peptide company **PeptiDream Inc.**, said that this was

an important, if unsurprising, finding. “To us this is not new, but this is the first time such a systematic analysis has been published and put out there publicly, and from that respect it is exciting to see the work

clearly make the point that macrocyclic molecules can target new space,” he said.

Whitty and his collaborators are now synthesizing small libraries of macrocycles to experimentally test their guidelines. “We consider the results of this analysis to be a hypothesis to be tested. If we can use them to design structures that are successful, then we will gain confidence these guidelines will be useful,” he said. He added that the guidelines should be refined and strengthened as more high-quality structures of macrocycle-protein interactions are published.

Terrett said that incorporating that information will be critical because the limited number of structures—and lack of any synthetic macrocycles in the set—is the major limitation of the analyses.

“This is a set of protein structures bound only by natural products, of which only a few are therapeutically useful, and even fewer have oral bioavailability,” he said. “This needs to be kept in mind when reaching conclusions—it is a good representative set when considering macrocycles interacting with proteins, but binding to proteins does not reveal any information about the drug-like nature of the compounds, which is a separate consideration.”

Proteins meet proteins

In contrast to Whitty’s relatively macrocycle compound-focused analysis, Kritzer’s team took a protein-centric approach. His group developed a computational method to analyze thousands of protein-protein interactions with the goal of identifying new leads for the design of cyclic or stapled peptides.

The tool, LoopFinder, analyzes protein-protein structures and identifies interaction surfaces that naturally involve a short loop of four to eight amino acids. Such an interface is a natural starting point for developing a cyclic peptide, which could be designed to mimic the natural interaction between a pair of targets of interest.

“The most original part of this work is that we had to define what loops were for ourselves,” said Kritzer, who noted that size range and additional structural properties including proximity to the

“It is exciting to see the work clearly make the point that macrocyclic molecules can target new space.”

—Patrick Reid, PeptiDream Inc.

“Developing macrocycles is a three-component problem—what do you want to target, how do you design a macrocycle to target it and which method can you use for synthesis?”

—Adrian Whitty,
Boston University

virtual alanine scanning of the loops to identify amino acid residues particularly critical for the stability of the protein-protein interaction.

This narrowed the list down to a set of 1,400 ‘hot loops’ predicted to be critical for maintaining protein-protein interactions. Indeed, some of the hot loops were found in regions known to mediate protein-protein interactions, providing retrospective experimental validation of the approach.

Kritzer is now developing loop-inspired cyclic peptides to prospectively test whether targeting computationally predicted hot loops can disrupt protein-protein interactions. Lokey and Terrett noted that it would not be a trivial matter to go from identifying a natural loop that facilitates a protein-protein interaction to developing a drug-like macrocycle or stapled peptide that could be cell permeable and disrupt that interaction.

Whitty emphasized that understanding the general principles underlying how natural loops facilitate protein-protein interactions goes hand in hand with understanding how to design macrocyclic compounds that can disrupt protein-protein interactions. “This work addresses the same fundamental questions as our own work but from a different point of view,” he said.

Lokey agreed. “I think these loop motifs may provide some ideas for the design of synthetic macrocycles, for example, the use of serine- or threonine-mediated backbone-to-side chain hydrogen bonds to effect

protein-protein interaction surface had to be taken into account.

This tool was used to analyze 19,657 structures in the Protein Data Bank and identified about 25,000 protein-protein interface loops. To hone in on those with the most relevance for drug discovery, the team performed

loops, or other internal hydrogen-bonding motifs that may not only help constrain the structure for binding but also facilitate membrane-permeable conformations.”

The LoopFinder algorithm is unpatented, and Kritzer plans to make the tool publicly available online in the next few months.

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Biogen Idec Inc. (NASDAQ:BIIB), Weston, Mass.
Boston University, Boston, Mass.
Circle Pharma Inc., San Francisco, Calif.
Ensemble Therapeutics Corp., Cambridge, Mass.
Nanyang Technological University, Singapore
PeptiDream Inc. (Tokyo:4587), Tokyo, Japan
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Tufts University, Medford, Mass.
University of California, Santa Cruz, Calif.

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This week in therapeutics

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

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer	Fatty acid synthase (FASN; FAS)	<p>Mouse studies suggest inhibiting FASN could help prevent tumor regrowth after antiangiogenic therapy. In mouse xenograft models of metastatic human breast cancer, antiangiogenics caused tumor regression, but tumor regrowth and metastasis accelerated following drug withdrawal. In mouse models of breast, colon and lung cancer, the FASN inhibitor Xenical orlistat given after withdrawal of antiangiogenic therapy decreased tumor regrowth and metastasis compared with vehicle. Next steps could include testing the strategy in additional cancer models.</p> <p>Roche and GlaxoSmithKline plc market Xenical, a reversible lipase inhibitor that also targets FASN, to treat obesity. At least two other companies have FASN inhibitors in Phase I or earlier testing to treat various cancers, obesity or HCV infection.</p> <p>SciBX 7(32); doi:10.1038/scibx.2014.945 Published online Aug. 21, 2014</p>	Unpatented; licensing status not applicable	<p>Sounni, N.E. <i>et al. Cell Metab.</i>; published online July 10, 2014; doi:10.1016/j.cmet.2014.05.022</p> <p>Contact: Nor Eddine Sounni, University of Liege, Liege, Belgium e-mail: nesounni@ulg.ac.be</p>
Cancer	Guanine nucleotide binding protein q polypeptide (GNAQ; Gαq); MAS-related GPR member X1 (MRGPRX1; GPCR; SNSR4)	<p><i>In vitro</i> and cell culture studies suggest the small molecule compound BIM-46187 blocks pathogenic GPCR signaling by targeting the GNAQ subunit. In human epithelial kidney and human skin cancer cells, BIM-46187 specifically inhibited activation of GNAQ in response to GPCR stimulation without also inhibiting other Gα proteins. In biochemical binding and competition assays, BIM-46187 trapped GNAQ in an inactive, nucleotide-free state. Next steps include optimizing the potency of BIM-46187.</p> <p>Ipsen Group has BIM-46187 in preclinical development for cancer.</p> <p>SciBX 7(32); doi:10.1038/scibx.2014.946 Published online Aug. 21, 2014</p>	Unpatented; licensing status not applicable	<p>Schmitz, A.-L. <i>et al. Chem. Biol.</i>; published online July 17, 2014; doi:10.1016/j.chembiol.2014.06.003</p> <p>Contact: Evi Kostenis, University of Bonn, Bonn, Germany e-mail: kostenis@uni-bonn.de</p>
Cancer	Stem cell factor receptor tyrosine kinase (c-Kit; KIT; CD117)	<p><i>In vitro</i> studies have identified selective inhibitors of the drug-resistant c-Kit D816V mutant that could help treat cancers that have become refractory to existing kinase inhibitors. <i>In vitro</i>, lead compounds in the series of 7-azaindole-based molecules inhibited c-Kit D816V with IC₅₀ values < 10 nM. In a human leukemia cell line expressing c-Kit D816V, lead compounds inhibited proliferation at submicromolar IC₅₀ values. Next steps could include testing the compound in mouse models of cancer.</p> <p>AB Science S.A. has the small molecule tyrosine kinase/c-Kit inhibitor masitinib in multiple Phase III trials to treat various cancers.</p> <p>Amgen Inc. and Takeda Pharmaceutical Co. Ltd. have motesanib diphosphate, a small molecule tyrosine kinase/c-Kit inhibitor, in Phase III testing to treat non-small cell lung cancer (NSCLC).</p> <p>At least four other companies have compounds that inhibit c-Kit in Phase II or earlier testing for various cancers.</p> <p>SciBX 7(32); doi:10.1038/scibx.2014.947 Published online Aug. 21, 2014</p>	Patent and licensing status unavailable	<p>Lee, S. <i>et al. J. Med. Chem.</i>; published online July 8, 2014; doi:10.1021/jm500413g</p> <p>Contact: Sungwoo Hong, Institute for Basic Science, Daejeon, South Korea e-mail: hongorg@kaist.ac.kr</p> <p>Contact: Soon-Sun Hong, Inha University, Incheon, South Korea e-mail: hongs@inha.ac.kr</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Liver cancer	Prolactin (PRL); PRL receptor (PRLR)	<i>In vitro</i> and mouse studies suggest increasing PRL signaling could help prevent hepatocellular carcinoma (HCC). In human and mouse liver cell lines, enhanced PRL-PRLR signaling decreased tumorigenesis-associated inflammation compared with no treatment. In mouse models of chemical-induced HCC, <i>Prl</i> deficiency led to greater tumor burden and invasiveness than normal <i>Prl</i> expression. In the HCC models, domperidone-induced <i>Prl</i> production decreased the number of precancerous lesions in the liver compared with no treatment. Next steps include a prospective study to determine whether use of antipsychotics and other therapies that increase serum PRL levels is associated with a decreased risk of liver cancer. The generic domperidone, an antagonist of dopamine D2 receptor and dopamine D3 receptor, is marketed to treat emesis and induce lactation by promoting PRL production by the pituitary gland. SciBX 7(32); doi:10.1038/scibx.2014.948 Published online Aug. 21, 2014	Unpatented; licensing status not applicable	Hartwell, H.J. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 21, 2014; doi:10.1073/pnas.1404267111 Contact: Arlin B. Rogers, Tufts University, North Grafton, Mass. e-mail: arlin.rogers@tufts.edu
Melanoma; breast cancer; cancer	Adenosine A _{2A} receptor (ADORA _{2A}); CTLA-4 (CD152); ecto- 5'-nucleotidase (NT5E; NT; CD73); programmed cell death 1 (PDCD1; PD-1; CD279)	Mouse studies suggest ADORA _{2A} inhibitors could be repurposed to treat cancers that express CD73, which catalyzes the conversion of AMP to adenosine. In mouse models of metastatic Cd73 ⁺ melanoma, an ADORA _{2A} inhibitor combined with an anti-Pd-1 or anti-Ctla-4 antibody decreased the number of lung metastases compared with vehicle or either antibody alone. In a mouse model of Cd73 ⁺ mammary cancer, the ADORA _{2A} inhibitor plus anti-Pd-1 antibody decreased the number of lung metastases and increased survival compared with either agent alone. Next steps include testing ADORA _{2A} inhibitors combined with other immunotherapies in preclinical cancer models. Kyowa Hakko Kirin Co. Ltd. markets Nouriasit istradefylline, a small molecule ADORA _{2A} antagonist, to treat Parkinson's disease (PD). Roche and Biotie Therapies Corp. have tozadenant (SYN115; SYN-115), a selective small molecule ADORA _{2A} antagonist, in Phase II testing to treat PD. Vernalis plc has the small molecule ADORA _{2A} antagonist V8144 in Phase I/II testing to treat attention deficit hyperactivity disorder (ADHD) and CNS disorders. The compound also is in Phase I testing to treat PD. SciBX 7(32); doi:10.1038/scibx.2014.949 Published online Aug. 21, 2014	Unpatented; licensing status not applicable	Mittal, D. <i>et al. Cancer Res.</i> ; published online July 1, 2014; doi:10.1158/0008-5472.CAN-14-0957 Contact: Mark J. Smyth, QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia e-mail: mark.smyth@qimrberghofer.edu.au
Prostate cancer	Androgen receptor (AR); prostate-specific antigen (KLK3; PSA)	<i>In vitro</i> studies have identified indole carboxamide-based AR inhibitors that could help treat prostate cancer. In human prostate cancer cell lines, including one that is resistant to Xtandi enzalutamide, members from a series of indole carboxamide-based AR inhibitors decreased PSA production and cell proliferation at nanomolar to low micromolar IC ₅₀ values. <i>In vitro</i> , the compounds were shown to target a region within the AR ligand-binding domain and not the androgen-binding domain. Next steps could include testing the lead compounds in animal models of prostate cancer. Medivation Inc. and Astellas Pharma Inc. market the oral AR antagonist Xtandi to treat prostate cancer. The drug also is in Phase III testing for breast cancer. AstraZeneca plc markets the oral nonsteroidal AR inhibitor Casodex bicalutamide to treat prostate cancer. Bayer AG markets the AR antagonist Androcur cyproterone acetate to treat prostate cancer. SciBX 7(32); doi:10.1038/scibx.2014.950 Published online Aug. 21, 2014	Patent and licensing status unavailable	Ban, F. <i>et al. J. Med. Chem.</i> ; published online July 25, 2014; doi:10.1021/jm500684r Contact: Artem Cherkasov, The University of British Columbia, Vancouver, British Columbia, Canada e-mail: artc@interchange.ubc.ca

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Prostate cancer	Lysine-specific demethylase 4A (KDM4A; JMJD2A); KDM4B (JMJD2B)	Cell culture studies suggest inhibiting KDM4A and KDM4B could help treat prostate cancer. In human prostate cancer cell lines, shRNA knockdown of <i>KDM4A</i> or <i>KDM4B</i> increased apoptosis compared with no alteration. In a human prostate cancer cell line, a KDM4A and KDM4B inhibitor decreased proliferation compared with no treatment and downregulated expression of androgen receptor-responsive genes. Next steps include developing optimized derivatives of the lead KDM4A and KDM4B inhibitor for treatment of prostate cancer. SciBX 7(32); doi:10.1038/scibx.2014.951 Published online Aug. 21, 2014	Provisional patent application filed; unavailable for licensing	Chu, C.-H. <i>et al. J. Med. Chem.</i> ; published online June 27, 2014; doi:10.1021/jm500249n Contact: Wen-Ching Wang, National Tsing-Hua University, Hsinchu, Taiwan e-mail: wawang@life.nthu.edu.tw Contact: Hsing-Jien Kung, University of California, Davis Comprehensive Cancer Center, Sacramento, Calif. e-mail: hkung@nhri.org.tw
Dermatology				
Dermatology	Annexin A1 (ANXA1); formyl peptide receptor 1 (FPR1)	Cell culture studies suggest inhibiting ANXA1 or its receptor FPR1 could help treat drug-induced adverse cutaneous reactions. In cultured keratinocytes, supernatants of peripheral blood mononuclear cells (PBMCs) from patients with drug-induced cutaneous reactions led to keratinocyte death, whereas PBMC supernatants from patients without drug-induced skin reactions had no effect. In the same assay, neutralizing antibodies against ANXA1 or FPR1 blocked supernatant-induced cytotoxicity in keratinocytes. Ongoing work includes identifying small molecules that antagonize FPR1-induced necroptosis. SciBX 7(32); doi:10.1038/scibx.2014.952 Published online Aug. 21, 2014	Patent application filed; available for licensing	Saito, N. <i>et al. Sci. Transl. Med.</i> ; published online July 16, 2014; doi:10.1126/scitranslmed.3008227 Contact: Riichiro Abe, Hokkaido University Graduate School of Medicine, Sapporo, Japan e-mail: aberi@med.hokudai.ac.jp Contact: Hiroshi Shimizu, same affiliation as above e-mail: shimizu@med.hokudai.ac.jp
Wounds	Olfactory receptor family 2 subfamily AT member 4 (OR2AT4)	Cell and tissue culture studies suggest activating OR2AT4 could help promote wound healing. In a human keratinocyte cell line, synthetic sandalwood odorants were shown to activate OR2AT4. In the cell line, siRNA against <i>OR2AT4</i> blocked the proliferation- and migration-promoting effects of a synthetic sandalwood odorant, whereas control siRNA did not. In <i>ex vivo</i> human skin cultures, the sandalwood odorant increased markers of wound healing compared with vehicle. Next steps could include screening for OR2AT4 agonists and evaluating them in animal wound healing models. SciBX 7(32); doi:10.1038/scibx.2014.953 Published online Aug. 21, 2014	Patent and licensing status unavailable	Busse, D. <i>et al. J. Invest. Dermatol.</i> ; published online July 7, 2014; doi:10.1038/jid.2014.273 Contact: Daniela Busse, Ruhr University Bochum, Bochum, Germany e-mail: daniela.busse@rub.de
Hepatic disease				
Hepatic disease	Periostin (POSTN)	Studies in mice and humans suggest inhibiting periostin could help treat hepatosteatosis. In mouse models of obesity and in patients with nonalcoholic fatty liver disease, hepatic POSTN levels were higher than those in lean control mice or normal subjects. In obese mice, <i>Postn</i> shRNA or a Postn-neutralizing mAb decreased hepatosteatosis compared with control shRNA or a control mAb. Researchers did not disclose next steps, which could include humanizing the Postn-neutralizing mAb and evaluating it in additional models of hepatosteatosis. SciBX 7(32); doi:10.1038/scibx.2014.954 Published online Aug. 21, 2014	Patent application filed covering use in hepatosteatosis; unavailable for licensing	Lu, Y. <i>et al. J. Clin. Invest.</i> ; published online July 8, 2014; doi:10.1172/JCI74438 Contact: Xiaoying Li, Shanghai Institute of Endocrinology and Metabolism, Shanghai, China e-mail: lixysibs@ac.cn Contact: Guang Ning, same affiliation as above e-mail: guangning@medmail.com

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Neurology				
Alzheimer's disease (AD)	β -amyloid (A β)	Mouse studies suggest glycosphingolipid-enriched exosomes could help treat AD. In the amyloid precursor protein (APP) transgenic mouse model of AD, continuous intraventricular injection of glycosphingolipid-enriched exosomes decreased pathogenic A β levels by up to 50% in the hippocampus and decreased A β plaque formation compared with vehicle. Next steps could include testing the therapeutic strategy in other animal AD models. SciBX 7(32); doi:10.1038/scibx.2014.955 Published online Aug. 21, 2014	Patent and licensing status unavailable	Yuyama, K. <i>et al. J. Biol. Chem.</i> ; published online July 18, 2014; doi:10.1074/jbc.M114.577213 Contact: Yasuyuki Igarashi, Hokkaido University, Sapporo, Japan e-mail: yigarash@pharm.hokudai.ac.jp
Alzheimer's disease (AD)	Sphingomyelin phosphodiesterase 1 acid lysosomal (SMPD1; ASM)	Studies in mice and human samples suggest inhibition of SMPD1 could decrease AD pathology. In human plasma samples, sphingomyelin levels were higher in patients with AD than in healthy subjects. In a mouse model of AD, heterozygous deletion or pharmacological inhibition of Smpd1 improved learning and memory performance and decreased brain β -amyloid (A β) levels compared with no deletion or vehicle treatment. Next steps could include developing an SMPD1 inhibitor with good oral bioavailability. SciBX 7(32); doi:10.1038/scibx.2014.956 Published online Aug. 21, 2014	Patent and licensing status unavailable	Lee, J.K. <i>et al. J. Exp. Med.</i> ; published online July 21, 2014; doi:10.1084/jem.20132451 Contact: Jae-sung Bae, Kyungpook National University, Daegu, South Korea e-mail: jsbae@knu.ac.kr
Epilepsy; seizures	Transient receptor potential cation channel subfamily C member 7 (TRPC7)	Mouse and cell culture studies suggest inhibiting TRPC7 could help prevent status epilepticus. In mice, knocking out <i>Trpc7</i> increased resistance to chemically induced status epilepticus and decreased resulting mortality compared with no genetic alteration. Next steps include determining the critical site of action for TRPC7 with a more refined genetic approach to ablate TRPC7 in specific neuronal populations. SciBX 7(32); doi:10.1038/scibx.2014.957 Published online Aug. 21, 2014	Patent and licensing status undisclosed	Phelan, K.D. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 21, 2014; doi:10.1073/pnas.1411442111 Contact: Fang Zheng, University of Arkansas for Medical Sciences, Little Rock, Ark. e-mail: zhengfang@uams.edu Contact: Lutz Birnbaumer, National Institute of Environmental Health Sciences, Research Triangle Park, N.C. e-mail: birnbau1@niehs.nih.gov
Huntington's disease (HD)	Microtubule-associated protein- τ (MAPT; tau; FTDP-17)	<i>In vitro</i> and mouse studies suggest depleting tau deposits could help treat HD. In a transgenic mouse model of HD, homozygous or heterozygous knockout of <i>Mapt</i> prevented or decreased formation of inclusions and decreased motor impairment compared with no alteration. Next steps could include testing the effects of tau-targeted therapeutics in animal models of HD. TauRx Pharmaceuticals Ltd. has the tau aggregation inhibitor TRx0237 in Phase III testing for Alzheimer's disease (AD) and cognitive dysfunction. At least seven other companies have tau-targeted therapeutics in Phase I or earlier testing. SciBX 7(32); doi:10.1038/scibx.2014.958 Published online Aug. 21, 2014	Patent and licensing status unavailable	Fernández-Nogales, M. <i>et al. Nat. Med.</i> ; published online July 20, 2014; doi:10.1038/nm.3617 Contact: José J. Lucas, Spanish National Research Council and the Autonomous University of Madrid, Madrid, Spain e-mail: jjlucas@cbm.csic.es

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Ophthalmic disease				
Glaucoma	Caspase-8 (CASP8; FLICE); toll-like receptor 4 (TLR4)	Rodent studies suggest inhibiting CASP8 could help treat glaucoma. In a mouse model of glaucoma, <i>Tlr4</i> and <i>Casp8</i> mRNA levels were increased and <i>Tlr4</i> knockout decreased intraocular pressure-induced retinal damage and <i>Casp8</i> levels compared with what was seen in wild-type controls. In the mouse model, intravitreal injection of a Casp8 inhibitor after ischemia onset decreased glaucoma-induced retinal damage and retinal ganglion cell death compared with no treatment. In a rat model of acute glaucoma, Casp8 inhibition also suppressed inflammasome activation. Next steps could include identifying a CASP8 inhibitor with drug-like properties. SciBX 7(32); doi:10.1038/scibx.2014.959 Published online Aug. 21, 2014	Patent and licensing status unavailable	Chi, W. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 14, 2014; doi:10.1073/pnas.1402819111 Contact: Yehong Zhuo, Sun Yat-sen University, Guangzhou, China e-mail: zhuoyh@mail.sysu.edu.cn Contact: Kang Zhang, University of California, San Diego, La Jolla, Calif. e-mail: kang.zhang@gmail.com

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			
Genomewide screen for identifying barriers to generation of human induced pluripotent stem (iPS) cells	A screen to identify barriers to the generation of human iPS cells could aid the development of optimized reprogramming protocols. A screen using shRNA libraries covering 19,527 human genes and next-generation sequencing identified targets that could impede the reprogramming of human fibroblasts into iPS cells. The screen identified 956 genes involved in processes including ubiquitination, cell adhesion and motility, and endocytosis as putative barriers to reprogramming. The screen also identified genes involved in transcription, chromatin regulation and dephosphorylation. Next steps could include evaluating how targeting these barriers can impact iPS cell reprogramming efficiency.	Patent and licensing status unavailable	Qin, H. <i>et al. Cell</i> ; published online July 17, 2014; doi:10.1016/j.cell.2014.05.040 Contact: Miguel Ramalho-Santos, University of California, San Francisco, Calif. e-mail: miguel.ramalho-santos@ucsf.edu Contact: Jun S. Song, same affiliation as above e-mail: songj@illinois.edu
Gold plasmonic chip for biomarker discovery and diagnosis of type 1 diabetes	An islet antigen microarray on a gold plasmonic chip could be useful for discovering biomarkers and diagnosing type 1 diabetes. The gold plasmonic chip is a multiplexed islet antigen microarray that consists of nanostructured gold islands on a glass surface, which are coated with a layer of polyethylene glycol and then printed with islet antigens. When tested with human serum samples, the chip detected islet-targeting autoantibodies from sample volumes of about 2 µL and distinguished between type 1 and type 2 diabetes with sensitivity and specificity comparable to those of a radioimmunoassay. Next steps include using the chip to discover biomarkers for type 1 diabetes and to understand disease pathogenesis and forming a company to commercialize the technology.	Patent applications filed; licensing status undisclosed	Zhang, B. <i>et al. Nat. Med.</i> ; published online July 13, 2014; doi:10.1038/nm.3619 Contact: Brian J. Feldman, Stanford University, Stanford, Calif. e-mail: feldman@stanford.edu Contact: Hongjie Dai, same affiliation as above e-mail: hdai1@stanford.edu
Immunosignature diagnosis platform	Immunosignature profiles could be used to diagnose or classify various human diseases. A microarray made of 10,000 random-sequence peptides binds antibodies in patient blood samples and produces a pattern of bound peptides that represent an immunosignature. In a 1,500-patient screen, the 10,000-peptide microarray classified 14 different types and stages of cancer with at least 95% accuracy. Next steps include using the platform for early detection of breast cancer.	Patented; licensed to HealthTell Inc.	Stafford, P. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 14, 2014; doi:10.1073/pnas.1409432111 Contact: Phillip Stafford, Arizona State University Biodesign Institute, Tempe, Ariz. e-mail: phillip.stafford@asu.edu
Chemistry			
Asparagine-aspartate peptide ligase for peptide macrocyclization	An asparagine-aspartate ligase, butelase 1, could enable the synthesis of cyclic peptides with utility for drug discovery. The enzyme was isolated from a tropical plant, <i>Clitoria ternatea</i> , and was shown to mediate the cyclization of non-native peptide substrates, including bioactive peptides. Next steps could include continued identification of plant-produced natural products with drug-like properties and adaptation of butelase 1 into a high throughput system for peptide cyclization and selection (<i>see Circling back to basics, page 10</i>).	Patent and licensing status unavailable	Nguyen, G.K.T. <i>et al. Nat. Chem. Biol.</i> ; published online July 20, 2014; doi:10.1038/nchembio.1586 Contact: James P. Tam, Nanyang Technological University, Singapore e-mail: jjtam@ntu.edu.sg

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Guidelines for the design of orally available macrocycles	A set of guidelines derived from an analysis of structures of macrocycles bound to proteins could be used to aid the design of orally bioavailable macrocycles that disrupt protein-protein interactions. The analysis honed in on high-quality structures with relevance to drug discovery, focusing on 22 distinct macrocycle-protein complexes encompassing 19 macrocycles and 13 proteins. The resulting structural guidelines for macrocycles suggest a range of ring sizes from 14–38 constituents and note desirable features including a substantial degree of unsaturation in the ring. Next steps include synthesizing libraries of macrocycles guided by these insights and experimentally testing their properties such as cell permeability (<i>see Circling back to basics, page 10</i>).	Unpatented; licensing status not applicable	Villar, E.A. <i>et al. Nat. Chem. Biol.</i> ; published online July 20, 2014; doi:10.1038/nchembio.1584 Contact: Adrian Whitty, Boston University, Boston, Mass. e-mail: whitty@bu.edu Contact: Sandor Vajda, same affiliation as above e-mail: vajda@bu.edu Contact: Dima Kozakov, same affiliation as above e-mail: midas@bu.edu
Computational models			
Computational prediction of loops at protein-protein interfaces using LoopFinder	A computational approach to predict loops at protein-protein interfaces could help identify interactions amenable to pharmacological targeting. The program, LoopFinder, searches structural databases for peptide loops at protein-protein interactions, and it identified 121,086 loops. These loops were further analyzed by virtual alanine scanning to identify those that could potentially be targeted to disrupt protein-protein interactions. Next steps include experimentally testing whether compounds that mimic these potentially targetable loop structures can be used to disrupt protein-protein interactions between new targets (<i>see Circling back to basics, page 10</i>).	Unpatented; licensing status not applicable	Gavenonis, J. <i>et al. Nat. Chem. Biol.</i> ; published online July 20, 2014; doi:10.1038/nchembio.1580 Contact: Joshua A. Kritzer, Tufts University, Medford, Mass. e-mail: joshua.kritzer@tufts.edu
Disease models			
<i>Calmodulin binding transcription activator 1 (Camta1)</i> -deficient mice as a model of ataxia and neurodegenerative disease	<i>Camta1</i> -deficient mice could be useful for identifying and evaluating therapeutic candidates to treat ataxia and neurodegenerative diseases. Mice with nervous system-specific deletion of <i>Camta1</i> developed severe ataxia and showed loss of Purkinje neurons and progressive cerebellar atrophy. In cerebellum tissue samples from these mice, genomic profiling and gene ontology analysis identified 203 dysregulated genes with 84 of them associated with neuronal or neuroprotective functions. Next steps could include evaluating the effects of existing therapies for neurodegenerative diseases in the mouse model.	Patent and licensing status unavailable	Long, C. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 21, 2014; doi:10.1073/pnas.1411251111 Contact: Eric N. Olson, The University of Texas Southwestern Medical Center, Dallas, Texas e-mail: eric.olson@utsouthwestern.edu
Drug platforms			
Creation of biological pacemaker from ventricular myocytes	A gene therapy approach to create a temporary biological pacemaker in the heart could help patients who are contraindicated for an electronic pacemaker because of transient issues such as an active infection. In pigs, minimally invasive percutaneous delivery of adenoviral vectors encoding T-box 18 (TBX18) to the heart resulted in the formation of pacemaker cells from ventricular myocytes. The resulting biological pacemaker did not increase arrhythmic risk and recapitulated the behaviors of a native sinoatrial node, including pacemaker activity that was responsive to circadian rhythms and changes in physical activity. Next steps include IND-enabling, long-term efficacy and safety studies.	Patent pending covering use of TBX18 as a biological pacemaker; licensed to undisclosed party	Hu, Y.-F. <i>et al. Sci. Transl. Med.</i> ; published online July 16, 2014; doi:10.1126/scitranslmed.3008681 Contact: Eugenio Cingolani, Cedars-Sinai Heart Institute, Los Angeles, Calif. e-mail: eugenio.cingolani@csmc.edu Contact: Eduardo Marbán, same affiliation as above e-mail: eduardo.marban@csmc.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
HIV genome excision from host cells using guide RNA (gRNA)-directed Cas9 genome editing	<i>In vitro</i> studies suggest gRNA-directed Cas9 genome editing could help treat HIV. In human microglia, perivascular macrophages and T cells latently infected with HIV, transfection with Cas9 plus gRNA that targets an HIV-1 long terminal repeat caused excision of proviral DNA and prevented reactivation of the latent virus. In HeLa-derived cells, the gRNA and Cas9 combination protected against infection with a new HIV clone without off-target effects. Next steps could include testing the approach in animal models of infection. SciBX 7(32); doi:10.1038/scibx.2014.968 Published online Aug. 21, 2014	Patent and licensing status unavailable	Hu, W. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 21, 2014; doi:10.1073/pnas.1405186111 Contact: Kamel Khalili, Temple University, Philadelphia, Pa. e-mail: kamel.khalili@temple.edu
Imaging			
3D, carbonized polyacrylonitrile scaffolds to promote bone tissue regeneration	3D, carbonized polyacrylonitrile scaffolds could be useful for promoting bone repair and regeneration. The scaffold is created by electrospinning polyacrylonitrile onto water, which then gets lyophilized, carbonized and modified into a 3D cylindrical structure. In mice with calvarial bone defects, bone grafts using the 3D, carbonized polyacrylonitrile scaffolds showed greater bone formation and regeneration than equivalent grafts that use a control 3D scaffold made from poly(L-lactic acid). Next steps could include evaluating bone grafts that use the carbonized polyacrylonitrile scaffolds in large animal models. SciBX 7(32); doi:10.1038/scibx.2014.969 Published online Aug. 21, 2014	Patent and licensing status unavailable	Ryu, S. <i>et al. Angew. Chem. Int. Ed.</i> ; published online July 7, 2014; doi:10.1002/anie.201403794 Contact: Byung-Soo Kim, Seoul National University, Seoul, South Korea e-mail: byungskim@snu.ac.kr Contact: Jyongsik Jang, same affiliation as above e-mail: jsjang@plaza.snu.ac.kr
Markers			
Gastric tumor classification system	Studies in patient samples have identified a classification system for human gastric cancers that could help guide treatment decisions. Molecular and genetic analyses of 295 primary tumor samples from untreated patients with gastric cancer identified four classes of gastric tumors. The classes are Epstein-Barr virus-positive tumors with DNA hypermethylation, microsatellite unstable tumors with high mutation rates, genomically stable tumors with frequent ras homolog gene family member A (RHOA) mutations or fusions, and tumors with chromosomal instability. Next steps include determining how to use biomarkers from each gastric cancer class to stratify patients for clinical trials. SciBX 7(32); doi:10.1038/scibx.2014.970 Published online Aug. 21, 2014	Unpatented; licensing status not applicable	Cancer Genome Atlas Research Network. <i>Nature</i> ; published online July 23, 2014; doi:10.1038/nature13480 Contact: Adam J. Bass, Dana-Farber Cancer Institute, Boston, Mass. e-mail: adam_bass@dfci.harvard.edu

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Medical Research Council	5			Domperidone	14	MRGPRX1	13		
MedImmune LLC	6			Dopamine D2 receptor	14	MT-102	3		
Medivation Inc.	14			Dopamine D3 receptor	14	N			
				E		Nourias	14		
				Ecto-5'-nucleotidase	14	NT	14		
				Enobosarm	1	NT5E	14		
				Enzalutamide	14	O			
				Etodolac	3	OHR/AVR118	3		
				F		Olfactory receptor family 2 subfamily AT member 4	15		
				FAS	13				
				FASN	13				
				Fatty acid synthase	13				

