

**THIS WEEK****ANALYSIS****COVER STORY****1 Channel-tuning neuropathy**

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A Brigham and Women's Hospital team has identified an enzyme that regulates white adipose plasticity, whereas a University of Cambridge team has identified a secreted protein that activates brown fat. Testing the potential of these new targets for boosting or activating calorie-burning brown fat will require developing lead therapeutic molecules.

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By Michael J. Haas, Senior Writer

An international team has shown that a peptide inhibitor of methylglyoxal treated hypersensitivity to pain in diabetic mice.<sup>1</sup> Although future studies could determine whether the mechanism plays a role in other types of neuropathic pain, the team is now seeking venture dollars to help spin out a new company based on the findings.

Painful diabetic neuropathy (PDN) is a subset of diabetic neuropathy that involves peripheral burning and tingling sensations and hypersensitivity to thermal or mechanical stimuli that are not ordinarily painful.

Hyperglycemia-induced vascular and neuronal changes are known to cause diabetic neuropathy, and past studies have linked neuronal hyperexcitability and increased sodium channel currents to PDN.<sup>2-4</sup> Nevertheless, the precise molecular mechanisms underlying PDN are not well understood.

One possible hyperglycemia-related culprit is methylglyoxal, a toxic by-product of glucose metabolism that has no biological function. In healthy people, it is converted to nontoxic D-lactate by glyoxalase 1 (GLO1), GLO2 and the cofactor glutathione.

In patients with diabetes, serum levels of methylglyoxal can be elevated due to hyperglycemia and deficiencies in GLO1 and glutathione. Previous studies also have shown that, compared with in other cell types, Glo1 is expressed at low levels in the peripheral neurons of normal rats and at even lower levels in those of diabetic rats,<sup>5</sup> and that the enzyme is necessary for neuronal integrity in roundworms (*Caenorhabditis elegans*).<sup>6</sup>

Collectively, those findings led an international team, which included researchers from the roundworm study, to hypothesize that methylglyoxal might induce changes in peripheral neuronal excitability to cause PDN.

The team first determined that type 2 diabetes patients with foot pain had higher plasma levels of methylglyoxal than diabetic patients without pain and nondiabetic control subjects.

Next, the researchers showed that diabetic mice and methylglyoxal-treated healthy mice exhibited hypersensitivity to thermal and mechanical stimuli, whereas untreated healthy controls did not. In peripheral neurons from the diabetic and methylglyoxal-treated mice, the metabolite had bound the sodium channel Nav1.8 (Pn3; Scn10a) in nociceptive (pain-sensing) neurons to increase excitability (*see Figure 1, "PDN: a sodium channel bound to excite"*).

The team also found a similar increase in Nav1.8 modifications in the peripheral nerves of patients with diabetes compared with peripheral nerves of nondiabetic subjects.

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Additional studies showed that *Nav1.8*-deficient mice treated with methylglyoxal did not experience thermal and mechanical hypersensitivity, thus confirming that metabolite modification of the sodium channel is responsible for the hypersensitivity.

Lastly, the researchers showed that a methylglyoxal-scavenging peptide decreased thermal and mechanical hypersensitivity in diabetic and methylglyoxal-treated mice compared with an inactive control peptide.

The half-life of the peptide's therapeutic effect in the mice was about 80 hours, suggesting that once-weekly administration in patients could be sufficient to treat PDN, team leader Peter Nawroth told *SciBX*. He added that reducing methylglyoxal to—or even below—normal physiological levels should pose no safety problems because methylglyoxal has no biological function.

Nawroth is professor of medicine, acting medical director and chairman of **Heidelberg University Hospital**. His team included researchers from the **University of Erlangen-Nuremberg**, the **University of Bucharest**, the **German Cancer Research Center**, **Riga Stradins University**, the **University of Latvia**, the **Psychiatric Center of South Wuerttemberg**, **The University of Warwick**, the **Albert Einstein College of Medicine of Yeshiva University**, the **Baker IDI Heart & Diabetes Institute**, the **University of Luebeck**, **Heinrich Heine University of Duesseldorf** and **The University of Tennessee College of Medicine**.

Additional team members from **Sanofi's Sanofi-aventis Deutschland GmbH** subsidiary and the reagent and instrument supplier **Biosistem d.o.o.** jointly performed some of the electrophysiological experiments in mouse neurons and the measurements of mechanical hypersensitivity in mice.

Data were reported in *Nature Medicine*.<sup>1</sup>

"These researchers are the first to demonstrate a link between elevated levels of methylglyoxal in diabetes and post-translational modifications of Nav1.8 to increase peripheral nerve excitability and induce hyperalgesia—both regarded as hallmark features of neuropathic pain," said Maree Smith, professor, reader and head of the pain research group at **The University of Queensland's** School of Pharmacy. Smith also is director of **TetraQ**, the university's preclinical drug development CRO.

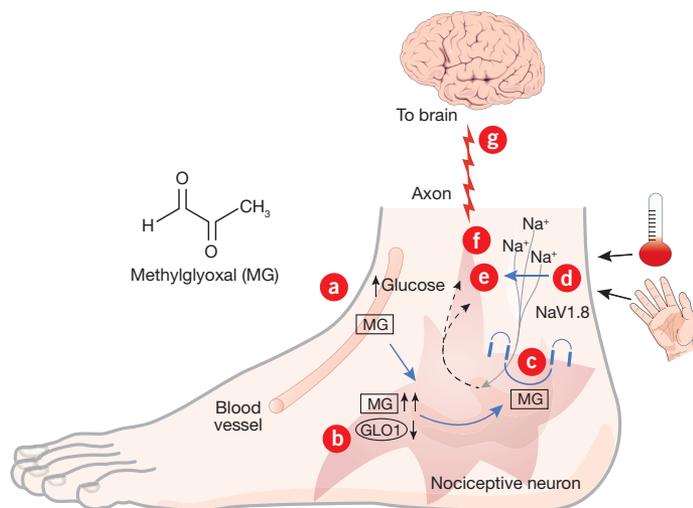
The study also demonstrates that "treatments that reverse diabetes-induced increases in methylglyoxal formation or that scavenge, degrade or clear the metabolite from the body could potentially treat or prevent painful diabetic neuropathy," she said.

Ru-Rong Ji, chief of pain research and professor of anesthesiology at **Duke University School of Medicine**, agreed.

"Treating neuropathic pain in diabetes patients is a major clinical challenge because current therapies are not very effective and have side effects," he said. "This *Nature Medicine* study offers new targets for treating such pain."

**"Treatments that reverse diabetes-induced increases in methylglyoxal formation or that scavenge, degrade or clear the metabolite from the body could potentially treat or prevent painful diabetic neuropathy."**

—Maree Smith,  
*The University of Queensland*



**Figure 1. PDN: a sodium channel bound to excite.** According to a study in *Nature Medicine*, methylglyoxal (MG) modifies sodium channel NaV1.8 (PN3; SCN10A) in nociceptive neurons to cause thermal and mechanical hypersensitivity in the extremities of patients with diabetes.

Diabetic hyperglycemia raises plasma levels of the metabolite MG [a] that accumulates in nociceptive and other peripheral neurons [b], which express only low levels of glyoxalase 1 (GLO1). The enzyme is required to convert MG to D-lactate.

In nociceptive neurons, MG binds the intracellular portion (inactivation gate) of NaV1.8 [c] and induces unknown structural modifications that inhibit the channel's ability to close in response to increases in the cell's membrane potential.

This increase allows thermal and mechanical stimuli [d] to induce excessive sodium influx [e], resulting in excessive neuronal firing [f] that causes pain [g], even in response to stimuli that are not painful in healthy individuals.

Z123212 (Z212), a small molecule that blocks NaV1.7 (SCN9A) and NaV1.8 sodium channels from **Zalicus Inc.**, is in preclinical development to treat chronic inflammatory and neuropathic pain.

Terry Snutch, CSO of **Zalicus Inc.**, concurred. "It is a nice piece of work with a new and interesting angle on how NaV1.8 could contribute to painful diabetic neuropathy. It also adds to the growing base of evidence that NaV1.8 is involved in neuropathic pain in general."

Later this year Zalicus expects to take its lead compound, Z160, an oral N-type calcium channel blocker, into Phase IIa testing to treat neuropathic pain that is not related to diabetes. The company also has Z123212 (Z212), a small molecule that blocks the NaV1.7 (SCN9A) and NaV1.8 sodium channels, and other undisclosed sodium channel blockers in preclinical development to treat chronic inflammatory and neuropathic pain.

The only two drugs approved to treat pain associated with diabetic neuropathy are Lyrica pregabalin and Cymbalta duloxetine. The former has side effects such as edema, weight gain, concentration and attention deficits and suicidal thoughts. The latter carries a black box warning for suicidal thoughts and behavior, and has other side effects such as hepatotoxicity and increased blood glucose in patients with diabetes.

**Pfizer Inc.** and **Eisai Co. Ltd.** market the  $\gamma$ -aminobutyric acid receptor (GABAR) agonist Lyrica to treat neuropathic pain, epilepsy and generalized anxiety disorder (GAD). **Eli Lilly and Co.** and **Shionogi & Co. Ltd.** market the selective serotonin and norepinephrine reuptake inhibitor (SSNRI) Cymbalta to treat diabetic peripheral neuropathic pain (DPNP), other types of chronic pain, anxiety and depression.

### Channel surfing

A key question now is whether methylglyoxal's role in pain is limited to diabetic neuropathy.

Ji said the metabolite's effects are probably most important in diabetes-related pain but that "it would also be interesting to know whether methylglyoxal alters NaV1.8 in other nondiabetic pain conditions," such as nerve trauma after surgery or neuropathy associated with viral infection and chemotherapy.

Smith said it is unlikely methylglyoxal-induced modification of NaV1.8 causes hypersensitivity in other types of neuropathic pain. The reason, she said, is that high levels of methylglyoxal develop as a result of hyperglycemia in diabetes.

Nevertheless, "it would be relatively straightforward to examine this question by comparing plasma levels of methylglyoxal in diabetes patients—as measured in this *Nature Medicine* study—with the levels in a range of animal models of neuropathic pain and in patients with neuropathic pain of differing etiologies" such as chemotherapy-induced neuropathy, post-herpetic neuralgia (PHN) and HIV/AIDS-associated neuropathy, she said.

If the team's methylglyoxal-scavenging approach turned out to be relevant only in PDN, "then the clinical hurdles to development of that approach could be high," said Zalicus CEO Mark Corrigan. "Diabetic neuropathy has multiple pathophysiologies, and that variability requires recruiting a large patient population for a clinical study—with associated costs that can be prohibitive for a therapy that has not shown clinical proof of concept in other types of neuropathic pain," he said.

He added that the variability in the pathophysiology underlying PDN has derailed a number of drugs in the clinic. "Consequently, the first indication for a clinical study of a neuropathic pain therapy is usually not diabetic neuropathy," he said.

Ji also wanted to know whether methylglyoxal altered other ion channels involved in diabetic pain, such as transient receptor potential ion channels.

Snutch agreed, adding that modulation of NaV1.8 is "probably not the only mechanism of pain in diabetic patients. For instance, high T-type calcium channel currents are associated with hyperalgesia" in the streptozotocin-induced diabetic mouse model used by Nawroth's team, he said.

Nawroth said the team is now optimizing and testing the methylglyoxal-scavenging peptide in additional animal models of diabetes.

To help develop the peptide as a PDN therapy, the team plans to collaborate with an undisclosed research group that has expertise at making peptides into orally available drugs, he said.

"We are also looking at the effect of methylglyoxal on other channels, not because they have the same importance as NaV1.8 in pain, but more to understand the crosstalk between channels" and to determine whether modifications to other channels are associated with other symptoms of diabetic neuropathy, he said.

Indeed, this week in *The Journal of Neuroscience*, researchers from the **University of Lausanne**, **University Hospital of Lausanne** and **University Hospital of Bicêtre** reported that low and/or abnormally distributed expression of potassium channel Kv1.2 (KCNA2) in sciatic nerve axons may contribute to PDN in diabetic mice and patients with diabetes.<sup>7</sup>

Nawroth said his team's findings are patented by Heidelberg University Hospital and are available for licensing or partnering.

However, "we are also looking for venture capital, as we would prefer to start a company" based on the findings rather than out-license the IP, he said.

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# Going to BAT

By Joanne Kotz, Senior Editor

Two teams are reporting new targets for inducing the conversion of white fat to a brown-like phenotype, or boosting the activity of existing brown fat, that could help treat obesity and related metabolic diseases. A **Brigham and Women's Hospital** team has identified an enzyme that regulates white adipose plasticity,<sup>1</sup> whereas a **University of Cambridge** team has identified a secreted protein that activates brown fat.<sup>2</sup>

White adipose tissue (WAT) stores energy as triglycerides, and brown adipose tissue (BAT) metabolizes fatty acids to generate heat. The mobilization of triglycerides by brown fat keeps in check the storage of fat by white fat, which helps control weight and overall metabolic status.

BAT was long known to exist in rodents and human infants, primarily as a defense against cold temperatures. In 2009, a trio of papers in *The New England Journal of Medicine* identified BAT in human adults and found that BAT activity was inversely correlated with BMI.<sup>3-5</sup>

At least one company is focused on targeting brown fat in obesity and other metabolic diseases. **Ember Therapeutics Inc.** has disclosed programs targeting bone morphogenetic protein 7 (BMP7; OP-1) and irisin, a secreted form of fibronectin type III domain containing 5 (FNDC5). Both proteins play a role in regulating brown fat development.

## Gut wrenching

Other groups have been looking for alternative pathways and targets to regulate brown fat formation and activation.

Results from a team led by Jorge Plutzky, director of the Vascular Disease Prevention Program at Brigham and Women's Hospital, suggest the enzyme aldehyde dehydrogenase 1 family member A1 (ALDH1A1) may be a target for inducing the formation of brown-like fat specifically in visceral white fat.

"Considerable attention is now being directed to new insights into different kinds of fat including subcutaneous versus visceral white fat, the latter being considered the

more pathogenic for cardiovascular disease and diabetes," said Plutzky.

Previous work from a Plutzky-led team showed knocking out *Aldh1a1* protected mice from high-fat diet-induced obesity.<sup>6</sup>

His team thus set out to determine whether ALDH1A1 also regulates body weight in humans.

In healthy people of normal weight, ALDH1A1 mRNA and protein levels were higher in visceral white fat than in subcutaneous white fat. In 40 people ranging from normal weight to morbidly obese, ALDH1A1 levels in visceral white fat correlated with BMI.

Next the team looked for the mechanism behind the connection.

*Aldh1a1*-deficient mice had higher expression of brown fat genes and greater mitochondrial activity in visceral white fat than wild-type mice. In contrast, expression of brown fat genes in subcutaneous white

fat and existing brown fat was relatively unchanged in the deficient mice.

Finally, the group tested the effects of antagonizing *Aldh1a1*. In obese mice, an antisense oligonucleotide against *Aldh1a1* decreased weight gain and visceral white fat mass and increased glucose and insulin tolerance compared with a control antisense oligonucleotide.

## Pump up your brown fat

Meanwhile, a team co-led by Andrew Whittle and Antonio Vidal-Puig identified a bone morphogenetic protein (BMP) family member, BMP8B, that ramps up thermogenesis in brown fat.

Vidal-Puig is a professor in the metabolic research laboratories at the University of Cambridge. Whittle is a postdoctoral research scientist in Vidal-Puig's laboratory.

Some BMPs are known to play a role in adipogenesis. For example, BMP2 and BMP4 drive white adipocyte formation, whereas BMP7 promotes brown adipocyte formation.

To determine whether BMP8B played a role in regulating fat, the Cambridge team studied mice and found that *Bmp8b* is predominantly expressed in the brain and in mature brown adipocytes.

The researchers next looked to see what role *Bmp8b* played in brown adipose tissue.

Mice exposed to cold had a 140-fold increase in *Bmp8b* expression in brown fat, whereas mice fed a high-fat diet had a fourfold increase. Both conditions activate brown fat.

Also, *Bmp8b*-deficient mice had lower body temperature and greater weight gain—despite reduced food intake—than wild-type mice. The researchers next treated brown adipocyte cultures with BMP8B, which increased stimulation-induced lipolysis compared with that seen in untreated controls.

Collectively, the findings suggested *Bmp8b* could play a role in mediating the activation of brown fat. The team went a step further and hypothesized that *Bmp8b* might be a central regulator of thermogenesis.

Indeed, intracerebroventricular injection of BMP8B led to greater neuronal innervation into BAT, higher core body temperature, lower body weight and better metabolic homeostasis than injection of vehicle.

## Therapeutics needed

The challenge for both targets will be developing safe therapeutic leads.

"Although early, we believe there is therapeutic potential of targeting both the ALDH1A1 and BMP8B pathways," said Lou Tartaglia, president and interim CEO of Ember.

ALDH1A1 is a member of a large family of aldehyde and retinaldehyde dehydrogenase enzymes, many of which perform essential functions. Thus, "the most significant challenge with ALDH1A1 is developing potent inhibitors that are specific enough and safe enough to treat metabolic disease," said Tartaglia.

"We are digging more into mechanisms while also exploring how to modulate the target," said Plutzky. "We have shown at least a proof of concept for antisense approaches, but small molecules may also be possible." He declined to disclose further details of therapeutic strategies the team will pursue.

"The challenge with BMP8B is that BMP family members are known to be involved in bone formation. The concern would be whether you have a therapeutic window where this could be used without inducing ectopic bone formation," said Bruce Spiegelman,

**"Considerable attention is now being directed to new insights into different kinds of fat including subcutaneous versus visceral white fat, the latter being considered the more pathogenic for cardiovascular disease and diabetes."**

—Jorge Plutzky,  
Brigham and Women's Hospital

professor in the Department of Cell Biology at the **Dana-Farber Cancer Institute** and a cofounder of Ember.

Whittle is not pursuing the development of a therapeutic BMP8B variant. Instead, his team is developing an assay that could be used to detect BMP8B in human serum and is working to identify the relevant receptor.

Plutzky declined to disclose the patent or licensing status of the work reported in *Nature Medicine*.

The results reported in *Cell* are unpatented, said Whittle.

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# Reprogramming heart failure

By Kai-Jye Lou, Staff Writer

Two groups at the **University of California, San Francisco** and **The University of Texas Southwestern Medical Center** have separately shown that delivering transcription factors directly to the heart reprogrammed cardiac fibroblasts and improved heart function in mouse models of post-myocardial infarction heart failure.<sup>1,2</sup> Because the approach acts directly on endogenous cardiac cells, it could avoid the problems associated with transplanting exogenous cells to improve heart function.

**LoneStar Heart Inc.** has licensed the UT Southwestern technology.

In 2010, the UCSF lab led by Deepak Srivastava had shown *in vitro* that retrovirus-mediated expression of three transcription factors—GATA binding protein 4 (Gata4), myocyte enhancer factor 2C (Mef2c) and T-box 5 (Tbx5)—reprogrammed mouse cardiac fibroblasts into cells with a cardiomyocyte phenotype.<sup>3</sup> They also showed that it was possible to transplant the reprogrammed cells into a mouse heart.

In the new studies, Srivastava's group at UCSF and Eric Olson's group at UT Southwestern have shown that it may be possible to avoid the transplantation step altogether by directly injecting the same transcription factors into the heart to trigger reprogramming of endogenous cardiac fibroblasts.

Srivastava is director of the **Gladstone Institute of Cardiovascular Disease**. He also is a professor in the Department of Pediatrics and the Department of Biochemistry & Biophysics at UCSF. Olson is professor and chairman of the Department of Molecular Biology at UT Southwestern and cofounder and chief scientific advisor to LoneStar Heart.

In both studies, the groups boosted the activity of the original three transcription factors—GATA4, MEF2C and TBX5—by delivering them to the heart in combination with additional agents.

In a mouse model of myocardial infarction (MI), the UCSF group first showed that injecting a vector encoding Gata4, Mef2c and Tbx5 into the heart led to direct conversion of cardiac fibroblasts into cardiomyocyte-like cells and improvement of cardiac function following injury.

The group then co-injected the same vector with thymosin  $\beta$ 4, which resulted in greater improvement of cardiac function in the mouse MI model than that seen using vector alone. Past studies led by Srivastava and others have suggested thymosin  $\beta$ 4 is cardioprotective and could promote cardiac repair and recovery of cardiac function.<sup>4-6</sup>

In its study, the UT Southwestern group showed that injecting the heart with a vector encoding GATA4, MEF2C and TBX5 plus a fourth transcription factor, heart and neural crest derivatives expressed 2 (HAND2), led to about fourfold more cardiomyocyte-like cells *in vitro* and quicker recovery of heart function *in vivo* than injection of a vector encoding only the original three transcription factors.

Results of both studies were published in *Nature*.

“The most interesting aspect of these studies—and where I think they could have the greatest impact—is in showing that you could regenerate the myocardium with the tissue that's already there,” said Patrick Most, head of the Center for Molecular and Translational Cardiology at **Heidelberg University**.

## Replacing transplantation

Having shown the feasibility of directly reprogramming endogenous cardiac fibroblasts into cardiomyocyte-like cells, the UCSF and UT Southwestern teams could bypass the challenges associated with cardiomyocyte transplantation.

Olson noted that the difficulties encountered with such therapies include transplanted cells “not engrafting efficiently, not surviving for the long term and not turning into heart muscle.”

Coaxing endogenous cardiac fibroblasts to convert into myocytes “would be easier than trying to deliver cells to the heart,” said Srivastava. “The endogenously generated myocytes would be more likely to integrate with the existing electrical and muscular network of the heart than transplanted cells.”

Moreover, cardiac fibroblasts “are present in excess in the heart and important for the initial phase of scar formation following injury. We thought that if even a small percentage of these cells could be repurposed to take on a myocyte role, we could enhance the function of injured hearts,” said Olson.

Roger Hajjar, director of the Cardiovascular Research Center and a professor of medicine at **Mount Sinai School of Medicine** and a cofounder of **Celladon Corp.**, said both groups' approaches provide a more direct pathway to generating cardiomyocytes.

“The route others have been using to generate these cells is first to turn fibroblasts into induced pluripotent stem cells and then to differentiate them into cardiomyocytes,” but cells generated in this manner will still need to be transplanted, he told *SciBX*.

Celladon's Mydicar, a recombinant adeno-associated viral (AAV) vector carrying the gene for ATPase Ca<sup>++</sup> transporting cardiac muscle slow twitch 2 (SERCA2A; ATP2A2), has completed a Phase I/II study in patients with advanced heart failure. The gene is a regulator of myocardial contractility.

## Fine-tuning expression

Key challenges moving forward include restricting the effects of the vector to the heart and improving the vector's transduction efficiency.

“In our experience with localized delivery of adeno-associated viral vectors, we found that about 40% of the vector still leaves the heart and transfects other cells,” Most told *SciBX*. Thus, the researchers will need to develop a way to control and restrict the expression of the vector-encoded genes to cardiac fibroblasts, he said.

Hajjar wanted to see further improvement in the vector's transduction efficiency, which on average was still under 10% based on the two papers.

“A higher transduction efficiency will probably be needed to convert a sufficient number of cardiac fibroblasts into myocytes that will then

“The most interesting aspect of these studies—and where I think they could have the greatest impact—is in showing that you could regenerate the myocardium with the tissue that's already there.”

—Patrick Most,  
Heidelberg University

be able to support the activity and generation of additional myocytes,” Hajjar told *SciBX*. “You would probably want transduction efficiencies to be in the 20%–30% range.”

Srivastava said the UCSF group is not only moving forward with its gene therapy approach in porcine models but also starting to screen for small molecules and secreted proteins that could activate the same gene networks as those activated by the transcription factors.

The UT Southwestern group is now trying to elucidate the mechanisms that contribute to the observed effects in the mouse model and develop an optimized therapeutic protocol for testing in large animal models of heart failure.

The group has not yet decided whether it will move forward with the gene therapy approach or with some other therapeutic modality.

“Ideally one would like to identify drug-like small molecules that could upregulate expression of the four transcription factors,” said Olson. “One might even consider combining such molecules with microRNAs to further improve the effect. This technology is still in its early days.”

Olson is also cofounder and chief scientific advisor of miRNA company **miRagen Therapeutics Inc.**

UCSF and UT Southwestern have separately filed patents to cover the findings described in their respective papers.

LoneStar Heart has exclusive worldwide rights to the UT Southwestern technology and is using it to guide the development of the company’s existing programs in restorative heart cell modulators and cellular and genetic therapies for advanced heart failure.

The UCSF patents are available for licensing.

Lou, K.-J. *SciBX* 5(22); doi:10.1038/scibx.2012.566  
Published online May 31, 2012

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#### COMPANIES AND INSTITUTIONS MENTIONED

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**Gladstone Institute of Cardiovascular Disease**, San Francisco, Calif.  
**Heidelberg University**, Heidelberg, Germany  
**LoneStar Heart Inc.**, Laguna Hills, Calif.  
**miRagen Therapeutics Inc.**, Boulder, Colo.  
**Mount Sinai School of Medicine**, New York, N.Y.  
**University of California, San Francisco**, Calif.  
**The University of Texas Southwestern Medical Center**, Dallas, Texas

# Sequencing human diversity

By Lev Osherovich, Senior Writer

A team led by researchers at **GlaxoSmithKline plc** and the **University of California, Los Angeles** has amassed the most extensive catalog to date of sequence variation in genes that encode drug targets.<sup>1</sup> Unraveling how these variants influence drug response and disease susceptibility will require phenotypic studies in model systems.

Previously, the GSK team assembled a large database of single-nucleotide polymorphisms across the genomes of healthy individuals.<sup>2,3</sup> That work painted a picture of human genetic diversity, but the microarray technology used in the study was not suited for identifying functional differences in disease-related genes.

To pinpoint those differences, the GSK team turned to a higher-resolution technique—DNA sequencing.

In the new study, the team sequenced the entirety of 202 drug target genes in a total of 14,002 cases and controls from a dozen cohorts of patients with cardiovascular, metabolic, autoimmune and neurological diseases.

The group expected to uncover a few key mutations present in patients with disease. Instead, they found many patients and controls harbored multiple genetic variants that differed from reference DNA sequences obtained from earlier sequencing efforts such as the Human Genome Project and the 1000 Genomes project.

“We asked what is the impact of rare genetic variants on disease in a large scale,” said team coleader Matthew Nelson, director of statistical genetics at GSK. “What we had hoped to find was a few genes with genetic variants that are associated with disease. In fact, most patients had a large variety of rare mutations.”

“We found surprising heterogeneity across drug target genes,” added Stephanie Chisoe, acting head of genetics at GSK.

The study was co-led by John Novembre, assistant professor of ecology and evolutionary biology at UCLA.

“The sheer quantity and abundance of rare variants sprinkled throughout the genome is a surprise,” said Joshua Akey, associate professor of genome sciences at the **University of Washington**.

Akey heads an academic consortium that has conducted a survey of genetic variation across the exome, the portion of the genome that encodes proteins.<sup>4</sup>

Whereas the GSK-UCLA team went deep into drug target genes in a large number of individuals, Akey’s team went wide and

sequenced 15,585 genes from 2,440 individuals. Both teams reported their findings concurrently in *Science*.

## Everybody is different

Nelson’s team sequenced 864 kilobase pairs and found that 1 in 17 nucleotides harbored at least 1 mutation compared with a reference sequence. The more subjects the team sequenced, the more variants they found. Extrapolating to a million individuals, the team predicted there would be up to 452 variants per kilobase pair of sequenced DNA.

Most of the variants found by the sequencing effort were rare—74% of the mutations were found in only 1–2 subjects. Moreover, 90% of the mutations had never been reported.

Some of the mutations were silent, but a fraction of them changed the amino acid sequence and presumably the function of the encoded protein. Indeed, 105 protein-altering variants in 73 genes were found in multiple individuals.

Akey said his own team’s analysis of the prevalence of rare genetic variants in the wider genome is in line with what the GSK and UCLA team saw. His team’s data predict the average individual’s exome contains 150 variants that deviate from the reference sequence.

## Variation information

The findings suggest most individuals are likely to carry a handful of rare genetic variants that affect the function in disease-associated proteins. However, the complex nature of diseases affected by these genes makes it difficult to predict how—and if—the mutations affect disease or drug susceptibility.

“The biggest challenge in the field is to show a causal relationship between these rare variants and disease,” said Akey. “Sometimes these variants can obviously affect the structure or function of proteins, but most of the time these effects will be not obvious.”

Nelson said the scale of his group’s study was not sufficient to prove that a particular gene variant causes disease or affects drug response. This is because the majority of the mutations are unique or too rare to support a statistical argument for causality.

Instead, Nelson and Chisoe think the best use of the data will be to guide the design of experiments to test how these variants affect protein function or drug response in preclinical disease models.

“There’s reason to believe that the functional impact of these rare variants can shed light on the action of drugs that hit these targets,” said Chisoe. “One possibility is to test the effect of the rare variants in a model system. Another idea is to look at how a drug works” on proteins encoded by the mutant genes.

As an example of how variation affects protein function, Nelson cited one of the genes sequenced by his team, *lipoprotein-associated phospholipase A<sub>2</sub>* (PLA<sub>2</sub>G7; PAFAH; Lp-PLA<sub>2</sub>). PLA<sub>2</sub>G7 is the target of GSK and **Human Genome Sciences Inc.**’s darapladib, which is in Phase III testing for atherosclerosis and coronary artery disease (CAD).

In an earlier pilot study of sequence variation in PLA<sub>2</sub>G7, Nelson’s team identified 8 rare variants in a cohort of 2,000 European individuals. *In vitro* studies showed that these variants reduced the enzyme’s activity.<sup>5</sup> Because homozygous carriers of the mutations have low PLA<sub>2</sub>G7 activity, the team predicted that those rare individuals would likely not benefit from darapladib treatment and should be excluded from trials of the compound.

Nelson and Chisoe said studying the disease phenotypes of individuals who carry such rare variants also could help validate potential drug targets, as mutations that reduce the protein’s function

“The biggest challenge in the field is to show a causal relationship between these rare variants and disease. Sometimes these variants can obviously affect the structure or function of proteins, but most of the time these effects will be not obvious.”

—Joshua Akey,  
University of Washington

might predict the effect of pharmacologically inhibiting those proteins.

Akey said that as more sequencing data roll in, the relationships between gene variants and diseases will become more apparent. If done with a sufficiently large number of individuals, direct sequencing could supersede indirect disease gene hunting methods like genomewide association studies.

“The simple approach is to collect a large number of patients and controls and ask whether variants in a particular gene correlate with disease,” said Akey. “There will be an avalanche of studies coming out in the next year on these relationships.”

Nelson said the sequence data from the study are not patented and will be made publicly available.

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Published online May 31, 2012

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#### COMPANIES AND INSTITUTIONS MENTIONED

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**Human Genome Sciences Inc.** (NASDAQ:HGSI), Rockville, Md.

**University of California, Los Angeles**, Calif.

**University of Washington**, Seattle, Wash.

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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	Casein kinase 1ε (CSNK1E; CKI-ε); c-Myc (MYC)	<i>In vitro</i> and mouse studies identified MYC-associated genes including <i>CSNK1E</i> that could be targeted to treat MYC-amplified cancers. In human fibroblasts overexpressing MYC, a screen identified genes encoding 148 proteins and microRNAs that were required for cell growth and survival. In mice with human MYC-driven neuroblastoma xenografts, small interfering RNA or a pharmacological inhibitor targeting CSNK1E, a protein encoded by a MYC-associated gene, decreased cell growth and tumor size compared with what was seen in neuroblastomas not driven by MYC. Next steps include developing an inhibitor against CSNK1E and validating the additional targets.  <b>SciBX 5(22); doi:10.1038/scibx.2012.568</b> <b>Published online May 31, 2012</b>	Patent applications filed; available for licensing	Toyoshima, M. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online May 23, 2012; doi:10.1073/pnas.1121119109 <b>Contact:</b> Carla Grandori, Fred Hutchinson Cancer Research Center, Seattle, Wash. e-mail: <a href="mailto:cgrandor@fhcrc.org">cgrandor@fhcrc.org</a>
Cancer	Endometrial differential 3 (EDI3)	Patient sample and <i>in vitro</i> studies suggest inhibiting EDI3 could help treat cancer. Researchers identified the enzyme EDI3, which cleaves glycerophosphocholine (GPC) to choline. Also, high levels of EDI3 were associated with low relapse-free survival in human patients with endometrial and ovarian cancer. In breast cancer cells, anti-EDI3 small interfering RNA increased GPC levels and decreased choline levels and cell migration compared with siRNA control. Next steps include identifying EDI3 inhibitors.  <b>SciBX 5(22); doi:10.1038/scibx.2012.569</b> <b>Published online May 31, 2012</b>	Findings patented; licensed to an undisclosed company	Stewart, J.D. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online May 8, 2012; doi:10.1073/pnas.1117654109 <b>Contact:</b> Jan G. Hengstler, Technical University of Dortmund, Dortmund, Germany e-mail: <a href="mailto:hengstler@ifado.de">hengstler@ifado.de</a>
Cancer	NADPH oxidase (NOX)	<i>In vitro</i> and mouse studies suggest inhibiting NOX could help treat cancer. In respiration-defective cancer cells, compared with respiration-competent cells, a NOX inhibitor increased cell death. In cancer cell lines with mitochondrial dysfunction, NOX inhibition increased cell death compared with that in cells without mitochondrial impairment. In mice with pancreatic cancer xenografts, the NOX inhibitor decreased tumor volume compared with vehicle control. Next steps include developing safer NOX inhibitors.  <b>SciBX 5(22); doi:10.1038/scibx.2012.570</b> <b>Published online May 31, 2012</b>	Patent status not applicable; unavailable for licensing	Lu, W. <i>et al. PLoS Biol.</i> ; published online May 8, 2012; doi:10.1371/journal.pbio.1001326 <b>Contact:</b> Peng Huang, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: <a href="mailto:phuang@mdanderson.org">phuang@mdanderson.org</a>
Cancer; sarcoma	Peptidyl arginine deiminase type IV (PADI4)	<i>In vitro</i> and mouse studies suggest PADI4 inhibitors could help treat sarcoma and other cancers. PADI4 is overexpressed in a wide range of human cancers. Chemical synthesis and subsequent testing in a human osteosarcoma cell line identified an amidine analog as a low micromolar inhibitor of PADI4. In mice with xenograft sarcoma tumors, the lead compound decreased tumor growth by >50% compared with vehicle. Also, the lead compound plus Zolinza vorinostat lowered tumor growth more than either agent alone. Ongoing work includes optimizing and testing the lead compound in additional animal models of cancer. Merck & Co. Inc. and Otsuka Pharmaceutical Co. Ltd. market the histone deacetylase (HDAC) inhibitor Zolinza to treat cutaneous T cell lymphoma (CTCL). The compound is in Phase III testing to treat mesothelioma and multiple myeloma (MM) and in Phase I testing to treat brain cancer.  <b>SciBX 5(22); doi:10.1038/scibx.2012.571</b> <b>Published online May 31, 2012</b>	Patented by Pennsylvania State University; available for licensing or partnering	Wang, Y. <i>et al. J. Biol. Chem.</i> ; published online May 17, 2012; doi:10.1074/jbc.M112.375725 <b>Contact:</b> Yanming Wang, Pennsylvania State University, University Park, Pa. e-mail: <a href="mailto:yuw12@psu.edu">yuw12@psu.edu</a> <b>Contact:</b> Gong Chen, same affiliation as above e-mail: <a href="mailto:guc11@psu.edu">guc11@psu.edu</a>

## This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Colorectal cancer	$\beta$ -Catenin (CTNNB1); forkhead box O3 (FOXO3; FOXO3a); phosphoinositide 3-kinase (PI3K); protein kinase B (PKB; PKBA; AKT; AKT1)	Patient and cell studies suggest inhibiting wingless-type MMTV integration site (WNT) and CTNNB1 signaling could help prevent drug resistance in colon cancer. In patient samples, high nuclear colocalization of CTNNB1 and the transcription factor FOXO3a, a downstream effector of PI3K and AKT signaling, was associated with a median survival of 25.7 months, whereas low nuclear colocalization of the 2 proteins was associated with a median survival of 33.7 months. In patient-derived colon cancer cells, high CTNNB1 levels were associated with increased resistance to PI3K and AKT inhibitors. In the patient-derived cells with high CTNNB1 levels, an inhibitor of WNT and CTNNB1 signaling reversed the resistance. Next steps include assessing nuclear CTNNB1 levels in patient colon cancer samples taken before and after treatment with a PI3K inhibitor.  <b>SciBX 5(22); doi:10.1038/scibx.2012.572</b> <b>Published online May 31, 2012</b>	Patent application filed covering nuclear CTNNB1 as a biomarker to predict response to FOXO3a-induced apoptosis by some targeted therapies; unavailable for licensing	Tenbaum, S.P. <i>et al. Nat. Med.</i> ; published online May 20, 2012; doi:10.1038/nm.2772 <b>Contact:</b> Héctor G. Palmer, Vall d'Hebron Institute of Oncology, Barcelona, Spain e-mail: <a href="mailto:hgpalmer@vhio.net">hgpalmer@vhio.net</a>
Head and neck cancer	FERM domain containing 4A (FRMD4A)	Patient sample and mouse studies suggest inhibiting FRMD4A could be used to treat head and neck squamous cell carcinoma (HNSCC). In human HNSCC samples, high FRMD4A levels correlated with increased risk of relapse. In a mouse xenograft model of HNSCC, <i>FRMD4A</i> knockdown decreased tumor growth and metastasis compared with normal expression of <i>FRMD4A</i> . Ongoing work includes further elucidating the mechanisms by which FRMD4A controls growth of HNSCC.  <b>SciBX 5(22); doi:10.1038/scibx.2012.573</b> <b>Published online May 31, 2012</b>	Patent application filed; available for licensing	Goldie, S.J. <i>et al. Cancer Res.</i> ; published online May 7, 2012; doi:10.1158/0008-5472.CAN-12-0423 <b>Contact:</b> Fiona M. Watt, Cambridge Research Institute, Cancer Research UK, Cambridge, U.K. e-mail: <a href="mailto:fiona.watt@cancer.org.uk">fiona.watt@cancer.org.uk</a>
Pancreatic cancer	Zinc and ring finger 3 (ZNRFF3); ring finger protein 43 (RNF43)	An <i>in vitro</i> study identified two new components of the wingless-type MMTV integration site (WNT) signaling pathway that could be targeted to treat pancreatic cancer or trigger tissue regeneration. Analysis of tissue microarray data identified ZNRFF3 and RNF43, two highly related E3 ubiquitin ligases, as negative regulators of WNT signaling. In human cells, antagonistic antibodies against the extracellular domain of ZNRFF3 increased WNT receptor levels and WNT signaling compared with antibody controls. Next steps include exploring the therapeutic potential of anti-ZNRFF3 antibodies in undisclosed regenerative medicine indications and testing inhibitors of WNT signaling in RNF43-mutated pancreatic tumors.  <b>SciBX 5(22); doi:10.1038/scibx.2012.574</b> <b>Published online May 31, 2012</b>	Patent status undisclosed; unavailable for licensing	Hao, H.-X. <i>et al. Nature</i> ; published online April 29, 2012; doi:10.1038/nature11019 <b>Contact:</b> Feng Cong, Novartis Institutes for BioMedical Research, Cambridge, Mass. e-mail: <a href="mailto:feng.cong@novartis.com">feng.cong@novartis.com</a>
<b>Endocrine/metabolic disease</b>				
Obesity	Bone morphogenetic protein 8b (BMP8B)	A study in cell culture and in mice suggests BMP8B could act both peripherally and centrally to help treat obesity. In brown adipocytes stimulated with norepinephrine, BMP8B led to a greater lipolytic response than no treatment. In mice, intracerebroventricular delivery of BMP8B increased thermogenic gene expression, core body temperature and weight loss compared with delivery of vehicle. Next steps include developing an assay to detect BMP8B in serum and identifying the relevant receptor ( <i>see Going to BAT, page 5</i> ).  <b>SciBX 5(22); doi:10.1038/scibx.2012.575</b> <b>Published online May 31, 2012</b>	Unpatented; licensing status not applicable	Whittle, A.J. <i>et al. Cell</i> ; published online May 11, 2012; doi:10.1016/j.cell.2012.02.066 <b>Contact:</b> Antonio Vidal-Puig, University of Cambridge, Cambridge, U.K. e-mail: <a href="mailto:ajv22@medschl.cam.ac.uk">ajv22@medschl.cam.ac.uk</a> <b>Contact:</b> Andrew J. Whittle, same affiliation as above e-mail: <a href="mailto:ajw232@cam.ac.uk">ajw232@cam.ac.uk</a>

## This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
<b>Infectious disease</b>				
Bacterial infections	Resolvin D1 (RvD1); RvD5	<p>A study in mice suggests RvD1 and RvD5 could be useful for treating bacterial infections. In a mouse model of bacterial infection, Rvd1 and Rvd5 each decreased bacterial titers and fever and increased survival compared with no treatment. Next steps could include testing RvD1 and RvD5 in other mouse models of bacterial infection.</p> <p>Resolvix Pharmaceuticals Inc. has resolvin analogs in preclinical development for inflammatory, pulmonary and cardiovascular diseases.</p> <p><b>SciBX 5(22); doi:10.1038/scibx.2012.576</b> Published online May 31, 2012</p>	Patent and licensing status undisclosed	<p>Chiang, N. <i>et al. Nature</i>; published online April 25, 2012; doi:10.1038/nature11042</p> <p><b>Contact:</b> Charles N. Serhan, Harvard Medical School, Boston, Mass. e-mail: <a href="mailto:cnserhan@zeus.bwh.harvard.edu">cnserhan@zeus.bwh.harvard.edu</a></p>
<b>Musculoskeletal disease</b>				
Bone repair	Semaphorin 3A (SEMA3A)	<p>Studies in cell culture and in mice suggest SEMA3A could be useful for bone repair. In murine cell culture, Sema3a inhibited differentiation of bone-resorbing osteoclasts, whereas vehicle did not. <i>Sema3a</i> knockout mice had greater osteoclast levels and lower bone density than wild-type controls. In an ovariectomized mouse model of postmenopausal osteoporosis, Sema3a decreased osteoclast activity and increased bone volume compared with saline controls. Next steps could include formulation and preclinical development of SEMA3A for bone loss indications.</p> <p><b>SciBX 5(22); doi:10.1038/scibx.2012.577</b> Published online May 31, 2012</p>	Patent and licensing status undisclosed	<p>Hayashi, M. <i>et al. Nature</i>; published online April 18, 2012; doi:10.1038/nature11000</p> <p><b>Contact:</b> Hiroshi Takayanagi, Tokyo Medical and Dental University, Tokyo, Japan e-mail: <a href="mailto:taka.csi@tmd.ac.jp">taka.csi@tmd.ac.jp</a></p>
<b>Neurology</b>				
Neurology	Claudin 5 (CLDN5)	<p>Mouse studies suggest blocking CLDN5 in the brain could help relieve brain edema and help treat traumatic brain injury. In mice, systemic delivery of <i>Cldn5</i>-targeting small interfering RNA one hour postinjury increased water permeability across the blood brain barrier, decreased edema and increased recovery of cognitive function compared with delivery of nontargeting siRNA. Ongoing studies include evaluating the safety of delivering the <i>Cldn5</i>-targeting siRNA in a nonhuman primate model. Avena Therapeutics Ltd. is developing a platform technology to modulate neuronal barrier permeability.</p> <p><b>SciBX 5(22); doi:10.1038/scibx.2012.578</b> Published online May 31, 2012</p>	Patent applications filed covering methods for modulating neuronal barrier; available for licensing from Avena Therapeutics	<p>Campbell, M. <i>et al. Nat. Commun.</i>; published online May 22, 2012; doi:10.1038/ncomms1852</p> <p><b>Contact:</b> Matthew Campbell, Trinity College Dublin, Dublin, Ireland e-mail: <a href="mailto:matthew.campbell@tcd.ie">matthew.campbell@tcd.ie</a></p>
Parkinson's disease (PD)	Leucine-rich repeat kinase 2 (LRRK2)	<p>Computational and <i>in vitro</i> studies identified a LRRK2 inhibitor that could be optimized to treat PD. High throughput screening, computational modeling and <i>in vitro</i> testing identified a lead diaminopyrimidine analog as a selective low nanomolar inhibitor of LRRK2. In normal mice, the compound showed good brain penetration. Future studies could include testing the compound in animal models of PD.</p> <p><b>SciBX 5(22); doi:10.1038/scibx.2012.579</b> Published online May 31, 2012</p>	Patent and licensing status unavailable	<p>Chen, H. <i>et al. J. Med. Chem.</i>; published online May 16, 2012; doi:10.1021/jm300452p</p> <p><b>Contact:</b> Dan Burdick, Genentech Inc., South San Francisco, Calif. e-mail: <a href="mailto:burdick.dan@gene.com">burdick.dan@gene.com</a></p> <p><b>Contact:</b> Huifen Chen, same affiliation as above e-mail: <a href="mailto:chen.huifen@gene.com">chen.huifen@gene.com</a></p>

## This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
<b>Transplantation</b>				
Graft-versus-host disease (GvHD)	Thrombomodulin (THBD; CD141)	<i>In vitro</i> and mouse studies suggest vitamin D-treated CD141 <sup>+</sup> dendritic cells (DCs) could induce immune tolerance and help treat GvHD. In cell culture, CD141 <sup>+</sup> dermal DCs induced immunosuppressive IL-10 expression and T <sub>reg</sub> cells, whereas CD141 <sup>-</sup> dermal DCs did not. In a mouse model of GvHD using transplantation of human peripheral blood mononuclear cells, vitamin D-induced CD141 <sup>+</sup> blood DCs decreased disease symptoms and increased mouse survival compared with untreated CD141 <sup>+</sup> blood DCs. Next steps could include testing vitamin D-induced CD141 <sup>+</sup> blood DCs in additional disease models of tolerance.	Patent and licensing status unavailable	Chu, C.-C. <i>et al. J. Exp. Med.</i> ; published online April 25, 2012; doi:10.1084/jem.20112583 <b>Contact:</b> Frank O. Nestle, King's College London and National Institutes for Health Research Biomedical Research Centre, London, U.K. e-mail: <a href="mailto:frank.nestle@kcl.ac.uk">frank.nestle@kcl.ac.uk</a>
		<b>SciBX 5(22); doi:10.1038/scibx.2012.580</b> Published online May 31, 2012		

## 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
<b>Assays &amp; screens</b>			
Cellular assay for detection of immune responses triggered by aggregated antibody therapeutics	<p>An <i>in vitro</i> assay using human peripheral blood mononuclear cells could help assess the immunogenic risk of aggregates formed by antibody therapeutics. Human IgG mAbs were generated and induced to form aggregates. In that assay, aggregated mAbs elicited innate and adaptive immune responses, whereas their monomeric counterparts did not. The assay was used to identify a cytokine signature that could help predict clinical immunogenic risk. Next steps include testing the effects of the aggregated therapeutics in a humanized <i>in vivo</i> model.</p> <p><b>SciBX 5(22); doi:10.1038/scibx.2012.581</b> Published online May 31, 2012</p>	Unpatented; unavailable for licensing	<p>Joubert, M.K. <i>et al. J. Biol. Cell</i>; published online May 14, 2012; doi:10.1074/jbc.M111.330902 <b>Contact:</b> Vibha Jawa, Amgen Inc., Thousand Oaks, Calif. e-mail: <a href="mailto:vjawa01@amgen.com">vjawa01@amgen.com</a> <b>Contact:</b> Marisa K. Joubert, same affiliation as above e-mail: <a href="mailto:mjoubert@amgen.com">mjoubert@amgen.com</a></p>
High throughput <i>human leukocyte antigen (HLA)</i> genotyping	<p>A high throughput <i>HLA</i> sequencing method could help identify disease-associated alleles. <i>HLA</i> gene polymorphisms are associated with susceptibility to infectious and autoimmune diseases. In 40 reference cell lines, an <i>HLA</i> genotyping method using single long-range PCR amplification and high throughput sequencing covered more polymorphic regions than existing approaches and matched 99% of the reported <i>HLA</i> genotypes. In 59 patient samples, the sequencing method identified 3 new <i>HLA</i> alleles. Ongoing studies are sequencing other important <i>HLA</i> genes.</p> <p><b>SciBX 5(22); doi:10.1038/scibx.2012.582</b> Published online May 31, 2012</p>	Stanford University filing patent applications; unavailable for licensing	<p>Wang, C. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online May 15, 2012; doi:10.1073/pnas.1206614109 <b>Contact:</b> Michael Mindrinos, Stanford University, Palo Alto, Calif. e-mail: <a href="mailto:mindrinos@stanford.edu">mindrinos@stanford.edu</a> <b>Contact:</b> Mark M. Davis, same affiliation as above e-mail: <a href="mailto:mmdavis@stanford.edu">mmdavis@stanford.edu</a> <b>Contact:</b> Ronald W. Davis, same affiliation as above e-mail: <a href="mailto:dbowe@stanford.edu">dbowe@stanford.edu</a></p>
Screen of sequentially administered cancer therapeutics to identify new combination therapies	<p>A screen of drug combinations could help identify new therapeutic combinations to help treat cancer. A cellular screen of combinations of sequentially dosed DNA-damaging agents showed that Tarceva erlotinib given four hours before doxorubicin led to a fivefold increase in apoptosis in a triple-negative breast cancer cell line compared with the combination dosed simultaneously. In mice, the timed combination decreased tumor growth compared with coadministration. Next steps include designing a clinical trial to test the sequential combination treatment and using the screen to look for other sequential combination therapies to treat cancer.</p> <p>Tarceva, an epidermal growth factor receptor (EGFR) inhibitor, is marketed by Astellas Pharma Inc., Chugai Pharmaceutical Co. Ltd. and Roche for non-small cell lung cancer (NSCLC) and pancreatic cancer.</p> <p><b>SciBX 5(22); doi:10.1038/scibx.2012.583</b> Published online May 31, 2012</p>	Patent application filed; available for licensing	<p>Lee, M.J. <i>et al. Cell</i>; published online May 11, 2012; doi:10.1016/j.cell.2012.03.031 <b>Contact:</b> Michael B. Yaffe, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: <a href="mailto:myaffe@mit.edu">myaffe@mit.edu</a></p>
<b>Disease models</b>			
Zinc finger nuclease (ZFN)-engineered human cell lines for probing the function of polypeptide N-acetylgalactosamine transferase (GalNAc) isoforms	<p>ZFN-engineered human cell lines that express polypeptide GalNAc transferase isoforms could help identify the role those enzymes play in disease. A human cell line was engineered to express a GalNAc transferase 2 (GALNT2; GalNAc-T2) isoform associated with dyslipidemia. In that cell line, O-glycosylation mediated by the GalNAc-T2 isoform inhibited activation of angiotensin-like 3 (ANGPTL3), which previous studies had linked to protecting against dyslipidemia. Next steps could include developing assays that probe the function of GalNAc transferases in other diseases.</p> <p><b>SciBX 5(22); doi:10.1038/scibx.2012.584</b> Published online May 31, 2012</p>	Patent and licensing status unavailable	<p>Schjoldager, K.T.-B.G. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online May 7, 2012; doi:10.1073/pnas.1203563109 <b>Contact:</b> Henrik Clausen, University of Copenhagen, Copenhagen, Denmark e-mail: <a href="mailto:hclau@sund.ku.dk">hclau@sund.ku.dk</a></p>

## This week in techniques

Approach	Summary	Licensing status	Publication and contact information
<b>Drug platforms</b>			
Generation of myeloid precursors from embryonic stem cells (ESCs) and induced pluripotent stem (iPS) cells	<i>In vitro</i> and mouse studies identified a method to differentiate ESCs and iPS cells into skeletal muscle cells to help treat muscular dystrophy. In cultured human ESCs or iPS cells, expression of paired box 7 (PAX7) generated myeloid precursors. In immunodeficient mice, transplantation of the myeloid precursors resulted in myofibers expressing human dystrophin (DMD). In a mouse model of muscular dystrophy, injection of myogenic precursor into muscle increased functional improvement of treated muscles compared with injection of saline control. Next steps include optimizing scalability and safety.  <b>SciBX 5(22); doi:10.1038/scibx.2012.585</b> <b>Published online May 31, 2012</b>	Findings unpatented; available for partnerships	Darabi, R. <i>et al. Cell Stem Cell</i> ; published online May 4, 2012; doi:10.1016/j.stem.2012.02.015 <b>Contact:</b> Rita C.R. Perlingeiro, University of Minnesota, Minneapolis, Minn. e-mail: <a href="mailto:perli032@umn.edu">perli032@umn.edu</a>
<i>In vivo</i> reprogramming of cardiac fibroblasts into cardiomyocytes	<i>In vivo</i> conversion of cardiac fibroblasts into cardiomyocytes could help treat heart damage. In mouse tail tip and cardiac fibroblasts, a retroviral vector expressing four transcription factors that control cardiac gene expression and development led to the conversion of the cells into cardiac-like myocytes. The four factors were GATA binding protein 4 (Gata4), heart and neural crest derivatives expressed 2 (Hand2), myocyte enhancer factor 2C (Mef2c) and T-box 5 (Tbx5). In a mouse model of myocardial infarction, intramyocardial injection of the same retroviral vector at 24 hours postinjury led to better cardiac function than injection of a control vector. Next steps include optimizing the treatment protocol and determining the mechanisms responsible for the efficacy. LoneStar Heart Inc. is developing a preclinical biologic to promote the development of cardiomyocytes in patients with congestive heart failure ( <i>see Reprogramming heart failure, page 7</i> ).  <b>SciBX 5(22); doi:10.1038/scibx.2012.586</b> <b>Published online May 31, 2012</b>	Patent applications filed; licensed to LoneStar Heart	Song, K. <i>et al. Nature</i> ; published online May 13, 2012; doi:10.1038/nature11139 <b>Contact:</b> Eric N. Olson, The University of Texas Southwestern Medical Center, Dallas, Texas e-mail: <a href="mailto:eric.olson@utsouthwestern.edu">eric.olson@utsouthwestern.edu</a>
<b>Markers</b>			
Genetic variation in drug target genes	A human genetic study suggests genetic variation in drug targets may influence patient disease susceptibility and drug response. The study involved sequencing 202 genes encoding established drug targets in 14,002 diseased and healthy subjects. Subjects had on average 111–153 variants per kilobase of sequenced DNA, and at least 90% of variants were rare and previously unknown. Next steps include testing the effects of the rare variants on disease risk and drug response ( <i>see Sequencing human diversity, page 9</i> ).  <b>SciBX 5(22); doi:10.1038/scibx.2012.587</b> <b>Published online May 31, 2012</b>	Unpatented; licensing status not applicable	Nelson, M.R. <i>et al. Science</i> ; published online May 17, 2012; doi:10.1126/science.1217876 <b>Contact:</b> Matthew R. Nelson, GlaxoSmithKline plc, Research Triangle Park, N.C. e-mail: <a href="mailto:matthew.r.nelson@gsk.com">matthew.r.nelson@gsk.com</a> <b>Contact:</b> John Novembre, University of California, Los Angeles, Calif. e-mail: <a href="mailto:jnovembre@ucla.edu">jnovembre@ucla.edu</a>

## Erratum: The Distillery: infectious disease: influenza virus

SciBX 5(20); doi:10.1038/scibx.2012.524  
Published online May 17, 2012

A Therapeutics item on infectious disease, highlighting an article by Ortigoza *et al.*, misstated the therapeutic target name. The target name in the target/marker/pathway column and throughout the summary should read “polymerase 1 (PB1).”



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