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By Lev Osherovich, Senior Writer

Researchers at **Harvard University** have discovered a hormone that promotes pancreatic islet  $\beta$  cell proliferation in mice.<sup>1</sup> **Evotec AG** and **Johnson & Johnson's** Janssen Pharmaceuticals Inc. unit have rights to the molecule and plan to turn it into a preclinical type 2 diabetes candidate this year. However, the hormone's relevance to disease in humans needs to be fleshed out.

$\beta$  Cells are the source of insulin, the hormone that controls blood sugar.  $\beta$  Cells die off in both type 1 and type 2 diabetes, albeit for different reasons—autoimmune destruction in the former, exhaustion in the latter.

Therapies for both forms of diabetes have largely focused on raising insulin production by what few  $\beta$  cells remain, but this approach brings diminishing returns in advanced cases of disease. The ideal approach would be to correct the metabolic or autoimmune problems that trigger the illness and then restore the full complement of  $\beta$  cells through regenerative therapy.

Now, a team led by Douglas Melton, professor of stem cell and regenerative biology at Harvard and co-director of the **Harvard Stem Cell Institute**, has identified the most potent means yet to stimulate  $\beta$  cell expansion—the liver-derived peptide hormone betatrophin.

“Betatrophin is a molecule that stimulates  $\beta$  cell proliferation, which is one attractive mechanism to expand  $\beta$  cell function,” said Evotec CSO Cord Dohrmann.

Previous studies by other teams have identified pathways and mechanisms that can modestly stimulate new  $\beta$  cell growth. These include peptide hormones such as glucagon-like peptide-1 (GLP-1)<sup>2</sup> and small molecule agonists of adenosine  $A_{2A}$  receptor (ADORA<sub>2A</sub>), as well as transdifferentiation approaches that convert other cell types into  $\beta$  cells.<sup>3</sup>

“When it comes to making new  $\beta$  cells, what sets this new work apart from prior work is the extent to which it stimulates cell proliferation,” said Dohrmann. “You can see two- to fivefold stimulation of proliferation with other pathways, but this causes a 10–20-fold increase in  $\beta$  cell numbers.”

“While there are other strategies for causing  $\beta$  cell replication, none of them are as potent and specific,” added Melton.

**Artificial resistance**

Prior studies by other researchers hinted that  $\beta$  cell mass and insulin levels could rise during insulin resistance, an early sign of type 2 diabetes.<sup>4</sup> To uncover why, Melton's team set up a mouse model in



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which insulin signaling was blocked with a pharmacological antagonist, leading to an artificial state of insulin resistance. The team then analyzed gene expression in a variety of tissues.

The group found that blocking insulin signaling led to the upregulation of betatrophin, a previously unknown 198-amino-acid secreted protein. The upregulation occurred in liver and fat tissues but not in the pancreas. Two other models for type 2 diabetes also showed higher betatrophin expression in the liver and fat than healthy controls.

The team reasoned that betatrophin relayed a signal from the liver and fat to the pancreas to compensate for insulin insensitivity. Indeed, transgenic overexpression of *betatrophin* in the liver led to a 17-fold increase in the rate of  $\beta$  cell replication in the pancreas compared with that seen in vector-treated controls.

Eight days after treatment, mice receiving extra betatrophin had three times as much  $\beta$  cell mass and produced about two times as much insulin. Those animals had lower fasting glucose levels and greater insulin sensitivity than vector-treated controls.

Results were published in *Cell*.

**“Betatrophin is a molecule that stimulates  $\beta$  cell proliferation, which is one attractive mechanism to expand  $\beta$  cell function.”**

**—Cord Dohrmann,  
Evotec AG**

## Insulin insult

The *Cell* findings help explain how the body adapts to dysregulation of insulin signaling, but questions remain about betatrophin's mechanism of action.

“Increased  $\beta$  cell mass in response to insulin resistance is a well-documented phenomenon,” said Hui Tian, VP of research at **NGM Biopharmaceuticals Inc.** “However, the factors or pathways that mediate this process are still unclear.”

Like Melton's team, NGM is trying to identify factors that raise  $\beta$  cell levels to treat metabolic disease. The company did not disclose whether it has identified betatrophin in its *in vivo* screens for  $\beta$  cell–boosting factors nor the identity of lead candidates in its  $\beta$  cell–regeneration collaborations with **Daiichi Sankyo Co. Ltd.** and **JDRF**, a not-for-profit group.

Olov Andersson, assistant professor in the Department of Cell and Molecular Biology at the **Karolinska Institute**, wanted to know whether betatrophin acted directly on  $\beta$  cells or on some other tissue.

“We have to determine whether it directly affects  $\beta$  cells,” said Andersson. “An alternative might be that there's a relay from another organ. To test this, you could isolate islets and treat them *in vitro*.”

Last year, Andersson and collaborators at the **University of California, San Francisco** reported that agonists of ADORA<sub>2A</sub> could promote  $\beta$  cell proliferation in zebrafish and mice.<sup>5</sup>

Another goal is to identify betatrophin's receptor, which may or may not be a known player in  $\beta$  cell proliferation.

“Betatrophin stands out from other  $\beta$  cell mitogens by being so potent,” said Andersson. “We don't know the downstream mediators, but these might be factors such as GLP-1,” which is another peptide hormone that promotes  $\beta$  cell proliferation.

**Beta strike**

As potent as betatrophin is in mice, the bigger question is whether the hormone will have an effect in diabetic humans.

“Even though we do know that betatrophin exists in humans, its relevance to human disease still needs to be demonstrated,” said Dohrmann.

Andrew Stewart noted that many compounds and targets for  $\beta$  cell proliferation have failed in the translational leap from mouse to man.

“There are now nearly a hundred different molecules that drive rodent cell replication that fail to do so in human  $\beta$  cells,” said Stewart. “The elephant in the room is: what about human  $\beta$  cells?” Stewart is professor of medicine, endocrinology, diabetes and bone disease and director of the Diabetes, Obesity and Metabolism Institute at the **Icahn School of Medicine at Mount Sinai**.

Stewart said one reason for the lack of success of prior strategies to boost human  $\beta$  cell replication is that mouse models typically involve young animals with robust capacity for pancreatic regeneration.

In contrast, typical patients with type 2 diabetes are middle-aged with heavily damaged pancreases that have exhausted the ability to naturally regenerate.

Stewart said several previous studies “have shown that as rodent  $\beta$  cells age, they become refractory to replication by things that work in juvenile rodents.”

Thus, it would be useful to test whether exogenous betatrophin can regenerate  $\beta$  cells in older rodents. Another key experiment would be to test the effect of purified recombinant betatrophin on cultured pancreatic islets obtained from diabetic cadavers.

Dohrmann and Melton said experiments along these lines are under way. Evotec and Melton’s team also are testing the effect of betatrophin on insulin response in a range of type 2 diabetes models.

Although the most obvious application of betatrophin is in type 2 diabetes, Melton thinks there is an opportunity to use the hormone to treat type 1 diabetes in conjunction with immunomodulatory therapy. He said between a patient’s initial diagnosis and the complete destruction of  $\beta$  cells, there is a window of opportunity to halt autoimmunity and regenerate the remaining  $\beta$  cells.

“My idea is that when you have a new-onset type 1 diabetes patient,

there’s still a lot of  $\beta$  cells,” said Melton. “A simple experiment is to combine betatrophin with an immunosuppressant.”

Another possibility is to administer betatrophin prophylactically in siblings of children with type 1 diabetes, who have a high risk of developing the disease.

Dohrmann said that betatrophin could be combined with DiaPep277, an immunomodulatory compound that Evotec has in Phase III testing for type 1 diabetes in partnership with **Clal Biotechnology Industries Ltd.** and **Teva Pharmaceutical Industries Ltd.**

The findings described in the *Cell* paper are patented by Harvard. The IP is licensed to Evotec and Janssen under a 2011 deal.<sup>6</sup> Dohrmann said betatrophin is the principal component of that deal, the details of which were not disclosed until now. Janssen will manufacture and test recombinant betatrophin in preclinical models, whereas Evotec will conduct *in vivo* and *in vitro* pharmacology

studies to identify betatrophin’s receptor and mechanism of action.

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**“While there are other strategies for causing  $\beta$  cell replication, none of them are as potent and specific [as betatrophin].”**

**—Douglas Melton,  
Harvard University**

# FGFR allosteric inhibition

By Lauren Martz, Staff Writer

Sanofi and collaborators at the **Flanders Institute for Biotechnology** at **Catholic University Leuven** have serendipitously discovered an allosteric inhibitor of the cancer target fibroblast growth factor receptor. The team hopes the allosteric mechanism will lead to improved safety over molecules that bind the receptor's active site and that the identification of this molecule will pave the way for designing other allosteric inhibitors—a process that has proven challenging.<sup>1,2</sup>

Fibroblast growth factor receptor (FGFR) is often mutated and overexpressed in cancers, in which it plays a role in angiogenesis. The FGFR family is made up of 4 different receptors, all of which have different splice variants, and 22 different ligands.

Although some FGFR signaling pathways contribute to cancer pathogenesis, others are important for normal physiological functioning. Nonspecific inhibition of FGFR signaling can cause serious side effects such as hyperphosphatemia.

Thus, inhibiting the target can cause both on- and off-target toxicities. In addition, FGFR inhibitors often have cross-reactivity with other receptor tyrosine kinases, which can lead to problems with blood pressure.

An even bigger challenge is that the FGFR family is large and structurally redundant, making it hard to target specific components. Indeed, competitive inhibitors of the ligand-binding sites may nonspecifically block binding of all ligands.

Sanofi and the Flanders Institute for Biotechnology (VIB) have found a potential solution by identifying an allosteric inhibitor of FGFR.

Allosteric inhibitors are difficult to design because it is often unclear which types of binding and which binding locations will noncompetitively alter the structure of the receptor. However, such compounds have the potential to improve specificity by targeting variable regions of closely related receptors, decreasing required drug doses and bypassing resistance to existing drugs.

In one study, a group led by Sanofi and VIB generated mouse disease data on an allosteric FGFR inhibitor, SSR128129E (SSR). In another paper, many of the same researchers studied the molecular structure of SSR and its mechanism of FGFR inhibition in more depth.

Both papers were published in *Cancer Cell*.

The team for the first paper was led by Jean-Marc Herbert, head of the Early-to-Candidate Unit at Sanofi, and Peter Carmeliet, professor of medicine and director of the Vesalius Research Center at VIB-Catholic University Leuven. The papers also included researchers from the **Yale School of Medicine**, **The University of Tokyo**, **the University of Liege**, **Goethe University Frankfurt**, **the Spanish National Cancer Research Center (CNIO)** and **Monash University**.

The researchers did not set out to find an allosteric inhibitor but

rather set up a high throughput screen designed to discover orthosteric FGFR inhibitors. In that screen, the team identified a compound that had low binding affinity for the FGFR ligand-binding site but nonetheless had high activity against FGFR ligand-driven endothelial cell proliferation.

This inhibitory pattern was not typical of orthosteric inhibition and suggested that the compound could act as an allosteric inhibitor.

To confirm that SSR inhibits FGFR allosterically, the group mapped its binding site and showed that SSR binds to the D3 extracellular domain of the receptor but not to the FGFR ligand-binding site located on the D2 domain.

In various cell lines, SSR inhibited FGFR. The compound had no effect on cancer cells lacking FGFR and did not alter the responses of other receptor tyrosine kinases to ligands.

Oral SSR plus a mAb targeting mouse Vegf receptor 2 (Kdr/Flk-1; Vegfr-2) also inhibited and delayed growth and decreased invasiveness and metastasis of Vegfr-resistant tumors in mice compared with either treatment alone. These findings suggest that FGFR allosteric inhibition could help overcome resistance to VEGFR inhibitors.

In a mouse model for arthritis, oral SSR decreased clinical severity, slowed disease progression and decreased the number of limbs affected by the disease compared with vehicle control. SSR blocked angiogenesis in the limbs, which decreased infiltration of inflammatory lymphocytes. These findings suggest the antiangiogenic effects of SSR could be applied to diseases other than cancer.

In the second paper, the goal was to confirm the allosteric binding mode of SSR. This paper also included researchers from the **University of Cambridge** and the **Hungarian Academy of Sciences**.

The team ruled out competitive binding with FGFR ligands, impaired dimerization of the receptor or ligands and general tyrosine kinase inhibition as SSR's mechanism. They also found that SSR binding to FGFR prevented internalization of the receptor into the cell, which could help inhibit downstream receptor signaling.

In binding experiments, the group showed that SSR bound all constructs that contained the D2 or D3 extracellular domains of FGFR, confirming that the compound interacted with the membrane receptor's extracellular domain.

2D NMR and crystallography studies pinpointed an extracellular binding site of an SSR-related compound on the FGFR D2 domain that was distinct from the known ligand or co-receptor binding sites. NMR and crystallography studies could not be used to identify the interaction of SSR with the D3 extracellular domain due to the dynamic, unfolded and disordered nature of the region.

Instead, Fourier transform infrared (FTIR) spectroscopy and *in silico* modeling were used to assess the effect of SSR on the D3 domain. These showed that the molecule induced a structural change in the domain that produced a new hydrophobic pocket on the receptor that may help alter ligand binding and receptor signaling.

"It has been very difficult to identify such molecules, and this sort of information could lead to direct screening programs for these types of

**"The work has a lot of possibilities for different therapeutic areas, and there are a lot of receptor tyrosine kinases implicated in cancer that could benefit from allosteric inhibition."**

—W. Mike Kavanaugh,  
Five Prime Therapeutics Inc.

compounds,” said W. Mike Kavanaugh, CSO and SVP of research at **Five Prime Therapeutics Inc.** “The work has a lot of possibilities for different therapeutic areas, and there are a lot of receptor tyrosine kinases implicated in cancer that could benefit from allosteric inhibition.”

Carmeliet added, “It will be useful to explore whether other receptor tyrosine kinases also have allosteric sites that can be targeted by small molecules. Sites exhibiting conformational flexibility are particularly interesting. This would require, however, a different and more demanding screening strategy than the traditional screening for orthosteric inhibitors, and it is here that the challenges lie.”

#### Allosteric advantage

Carmeliet told *SciBX* that “Sanofi has developed next-generation derivatives of SSR for further clinical development.” Herbert said the SSR analogs are in preclinical development. The company is not disclosing a timeline for advancing to the clinic.

Meanwhile, Harald Schwalbe, professor in the Institute of Organic Chemistry and Chemical Biology at Goethe University Frankfurt and corresponding author on the second paper, said his team is supporting the development of Sanofi’s molecules by studying the mode of molecular recognition of the compounds and completing SAR studies.

“The most obvious potential value of this compound would be to overcome the acquired and *de novo* resistance to currently available FGFR inhibitors,” said David McConkey, director of urological research and professor of cancer biology and urology at **The University of Texas MD Anderson Cancer Center**. “Effective targeting of receptor tyrosine kinases can lead to the accumulation of mutations that prevent drugs from inhibiting them. This new compound could completely bypass such mechanisms.”

Neil Thompson, SVP of biology at **Astex Pharmaceuticals Inc.**, agreed. “What is especially important is that this group has identified a completely new way to block the receptor,” he said. “Many resistance mutations occur within the target, and they are generally quite specific to the drug. Another site of activity is a great way to tackle resistance. We must give credit to this group, which has clearly done something very difficult.”

Astex and the Janssen Pharmaceuticals N.V. subsidiary of **Johnson & Johnson** have the pan-FGFR inhibitor JNJ42756493 in Phase I testing to treat cancers.

Thompson said that JNJ42756493 was designed to avoid cross-reactivity with VEGFR-2 and that the compound is currently being tested for effects on phosphatase homeostasis.

Carmeliet thinks SSRs’ allosteric mechanism could circumvent some of the safety issues associated with FGFR inhibition.

“Orthosteric inhibitors block ligands that activate the receptors. If tumors upregulate the ligands, then higher doses of orthosteric inhibitors must be used to competitively antagonize the ligands,” he said. “Such high doses of the inhibitors can cause toxic side effects for healthy tissues, as is the case with orthosteric VEGF inhibitors. By contrast, an allosteric inhibitor acts independently of the ligand concentration and is therefore safer to use as it cannot be overdosed.”

**“What is especially important is that this group has identified a completely new way to block the receptor.... We must give credit to this group, which has clearly done something very difficult.”**

**—Neil Thompson,  
Astex Pharmaceuticals Inc.**

Regardless of how a molecule blocks FGFR signaling, toxicity could still be an issue because it is not yet clear which downstream FGFR signaling pathways are selectively blocked by SSR.

Kevin Baker, VP of preclinical development at Five Prime, said SSR is still a general inhibitor of FGFR and still blocks most downstream signaling of the receptors, so there is the potential for related side effects such as elevated blood phosphate.

Carmeliet countered that SSR does not block all downstream FGFR signaling, as orthosteric inhibitors normally do, but acknowledged that the specific effects still need to be characterized.

Five Prime and **GlaxoSmithKline plc** are developing 3052230, an FGF ligand trap that selectively blocks cancer-associated FGF ligands while only weakly binding hormone-associated FGFs, which can result in toxicity.

Carmeliet told *SciBX* that the team is trying to identify new allosteric inhibitors for other receptor tyrosine kinases.

Sanofi and VIB have joint patents covering their screening method, and Sanofi has patented SSR and related analogs. The IP is not available for licensing.

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# Add radioactivity to the *Listeria*

By Michael J. Haas, Senior Writer

An Albert Einstein College of Medicine of Yeshiva University team has used radiolabeled *Listeria monocytogenes* to efficiently treat metastatic pancreatic cancer in mice.<sup>1</sup> Future studies could involve comparing or combining the strategy with existing nonradioactive immunotherapies that utilize the bacterium as a vector.

*L. monocytogenes* is a food-borne pathogen that can infect a range of mammalian cell types and cause potentially fatal illness (listeriosis) in immune-compromised individuals. For the last decade, attenuated strains of the bacterium have served as vectors for delivering antigens with high specificity to dendritic cells, thereby eliciting an immune response to cancer or infection.<sup>2</sup>

At least two companies—Advaxis Inc. and Aduro BioTech Inc.—have vaccines or immunotherapies based on attenuated *L. monocytogenes* in development to treat various cancers and infectious diseases.

Another advantage of the bacterium is that it survives longer—and thus replicates to a greater extent—in the immunosuppressive environment of tumors than in normal tissues. This has allowed it to be used for cancer imaging in animal models<sup>3</sup> and for expressing enzymes capable of converting chemotherapy prodrugs into active therapeutic agents in human breast cancer and mouse melanoma cell lines.<sup>4</sup>

Moreover, a 2009 study co-led by Claudia Gravekamp and Yvonne Paterson showed that an attenuated *L. monocytogenes* itself had efficacy in cancer. The bacterium decreased primary and metastatic tumor growth in mouse models for breast cancer almost as effectively as the same bacterial vector expressing a cancer antigen.<sup>5</sup>

Gravekamp is associate professor of microbiology and immunology at Albert Einstein College of Medicine. Paterson is professor of microbiology at the Perelman School of Medicine at the University of Pennsylvania, professor of nursing and associate dean for research at the University of Pennsylvania's School of Nursing and cofounder of Advaxis.

Those 2009 findings prompted a new team, co-led by Gravekamp and Ekaterina Dadachova, to start thinking about how to boost the tumoricidal activity of *L. monocytogenes* rather than using it as an antigen-delivery vehicle.

Dadachova, professor of radiology and of microbiology and immunology at Albert Einstein College of Medicine, proposed giving *L. monocytogenes* a radioactive payload.

In a mouse model for murine pancreatic cancer, an attenuated strain of *L. monocytogenes* replicated efficiently in metastatic tumors, poorly in primary tumors and not at all in normal tissues such as the spleen.

Next, the team linked an anti-*Listeria* antibody labeled with the radionuclide rhenium-188 (<sup>188</sup>Re) to the surface of the attenuated

*L. monocytogenes*. The team chose <sup>188</sup>Re because its half-life of about 17 hours allowed the delivery of a therapeutic dose of radiation in a relatively short time frame.

In the pancreatic cancer models, *L. monocytogenes* loaded with the largest quantity of <sup>188</sup>Re—dubbed RL-200—decreased the number of metastases in liver and other organs by about 90%, and the unlabeled bacterium decreased the number by about 50%, compared with vehicle or <sup>188</sup>Re alone. In primary tumors, RL-200 and the unlabeled bacterium decreased growth by about 64% and 20%, respectively.

Histopathological analyses showed that RL-200 did not cause damage to liver, kidneys or other normal tissues.

Data were reported in the *Proceedings of the National Academy of Sciences*.

Collectively, the results demonstrate that “radioactive *Listeria* could be valuable as an early second-line therapy—following removal of the primary pancreatic tumor—to treat existing metastases and prevent the development of new metastases,” Gravekamp told *SciBX*.

She added that RL-200 should also be able to eliminate any remaining primary tumor cells in patients because those cells would be actively dividing. In contrast, the primary tumors in the mouse models—in which RL-200 replicated poorly—were dormant.

“Using *Listeria* to deliver a payload to tumors is a clever idea,”

Paterson said. In the 2009 study, “we looked at *Listeria* alone and showed its effects on tumor growth and the mechanism by which it kills tumors. But we know this isn't always very effective, maybe because not all cancer cells get infected or contain enough of the bacteria to kill them. Adding a radionuclide would help because uninfected tumor cells that surround the *Listeria*-infected cell could be killed by radiation.”

## Radiotherapy, vaccine or both?

It is not yet clear whether *L. monocytogenes* will have better efficacy as a vehicle for delivering

radioactive payloads or as a vector for expressing cancer antigens in immune cells. Ultimately, it might be possible to combine both uses of the bacterium into one product.

Paterson said the two therapeutic approaches could be assessed with a “side-by-side comparison in the same tumor model. It would also be interesting to see a therapeutic that harnessed all three ways in which *Listeria* can be used against cancer: direct killing of tumor cells, delivery of a therapeutic payload to tumors and delivery of cancer antigens to immune cells.”

Steven Bodovitz, Aduro's associate director for strategic development, said combining radiotherapy and immunotherapy could be effective, based on results the company saw in a Phase I trial of its CRS-207 (ANZ-207). The product is a vaccine based on a live, attenuated strain of *L. monocytogenes* that expresses human mesothelin.

In the trial, two of three patients with non-small cell lung cancer (NSCLC) received the vaccine and subsequently received local radiotherapy intended to be palliative. “Both subjects demonstrated a systemic antitumor response that indicated synergy between” the two types of therapy, he said.

**“It would also be interesting to see a therapeutic that harnessed all three ways in which *Listeria* can be used against cancer: direct killing of tumor cells, delivery of a therapeutic payload to tumors and delivery of cancer antigens to immune cells.”**

—Yvonne Paterson,  
University of Pennsylvania

Thus, although Aduro and the PNAS team took differing approaches to combining *L. monocytogenes* and radiotherapy in the treatment of cancer, “we agree that the combination of radiotherapy and immunotherapy has the potential to be synergistic,” Bodovitz said.

CRS-207 also is in Phase II testing to treat pancreatic cancer and Phase I to treat malignant pleural mesothelioma. The company will present results from the Phase II trial this August at the annual meeting of the **American Society of Clinical Oncology**.

Aduro CSO Thomas Dubensky Jr. cautioned that combining immunotherapy and radiotherapy into one attenuated strain of *L. monocytogenes* might be challenging because different strains are not equally suited to both purposes.

For example, he said, “our attenuated strain of *Listeria* was designed to induce an immune response by targeting dendritic cells and other phagocytic cells of the immune system, not deliver a toxic payload directly to cancer cells.”

Nevertheless, Gravekamp said it should be possible to develop a single *Listeria*-based therapeutic that delivered antigens to immune cells and a radioactive payload to tumor cells. “A chemotherapeutic payload would probably kill the immune cells it infects, but our radionuclide might not,” she said.

She added that the team already has shown that RL-200 does not kill myeloid-derived suppressor cells—a type of immune cell involved in wound repair, inflammation and cancer.

However, she said, “we are definitely more interested in combination therapies that use RL-200 and a separate cancer vaccine because one type of therapy will not be sufficient against metastatic cancer.”

It would also be important to establish that *L. monocytogenes* is safe when delivering a therapeutic payload, said Robert Fine, associate professor of medicine and director of experimental therapeutics at the Herbert Irving Comprehensive Cancer Center at **Columbia University**.

“Even though the bacterium is attenuated, it’s still live and might be able to cause a dangerous, even fatal, infection,” he said.

The use of *L. monocytogenes* as a vector in cancer vaccines does not

**“We are definitely more interested in combination therapies that use RL-200 and a separate cancer vaccine because one type of therapy will not be sufficient against metastatic cancer.”**

**—Claudia Gravekamp,  
Albert Einstein College of Medicine  
of Yeshiva University**

raise the same concern because those bacteria do not survive long enough to replicate, he said.

Bodovitz agreed that additional studies are needed to establish the safety of the radioactive *Listeria*. He also wanted to see survival data for RL-200 in the animal models and know more about the agent’s mechanism of action.

Additionally, Fine said RL-200 should be tested in models for metastatic pancreatic cancer—such as those expressing mutant *K-Ras* and *p53*—that better correlate with human disease.

Gravekamp said the team will next test RL-200 in at least two xenograft models for metastatic pancreatic cancer.

Albert Einstein College of Medicine has patented the PNAS findings, and the IP is available for licensing. “We are very interested in finding an industry partner to take this approach into the clinic,” said Gravekamp.

Haas, M.J. *SciBX* 6(17); doi:10.1038/scibx.2013.407  
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#### COMPANIES AND INSTITUTIONS MENTIONED

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# CTCs gain character(ization)

By Tracey Baas, Senior Editor

Despite the recent shuttering of circulating tumor cell diagnostic company On-Q-ity Inc., researchers at **Massachusetts General Hospital** think there is still reason to innovate in the space. The group has developed a microfluidic platform able to identify circulating tumor cells that lack epithelial cell adhesion molecule.<sup>1</sup>

**Johnson & Johnson** has rights to the technology and is funding development of the product, which is dubbed CTC-iChip, under a 2011 deal with MGH.<sup>2</sup>

Circulating tumor cells (CTCs) are rare cells that break off from solid tumors and flow in the bloodstream. The CellSearch CTC Test from Johnson & Johnson's Veridex LLC unit is the only FDA-approved

product that detects the cells. According to CellSearch's label, a count of 5 or more CTCs per 7.5 mL of blood—the standard volume of blood drawn in the clinic—is predictive of shorter progression-free survival (PFS) and overall survival (OS) in patients with metastatic breast cancer or metastatic prostate cancer. A CTC count of three or more is associated with lower PFS and OS in patients with metastatic colorectal cancer.

However, CellSearch misses CTCs that are negative for epithelial cell adhesion molecule (EpCAM).

Isolating EpCAM<sup>-</sup> CTCs is particularly important because cancer cells downregulate expression of EpCAM as they proceed through epithelial-mesenchymal transition (EMT), a characteristic of metastasis, and there are multiple nonepithelial cancers such as melanoma.

Previous work from MGH developed CTC-iChips with surfaces modified with anti-EpCAM antibodies to bind EpCAM<sup>+</sup> CTCs.<sup>3,4</sup> However, only 1–2 mL of blood could be processed an hour, only EpCAM<sup>+</sup> CTCs could be isolated and the chip required a 3D image-scanning system because CTCs are immobilized on the chip surface.

Now, the MGH team has developed a next-generation chip with

**Figure 1. Isolating CTCs.** The circulating tumor cell (CTC)-iChip uses magnetic beads to label cells in whole blood. The blood sample is loaded onto the chip, on which lateral displacement, inertial focusing and magnetophoresis separate and collect the cells of interest from the bulk.

(I) Positive selection incorporates epithelial cell adhesion molecule (EpCAM)-targeting magnetic beads to bind CTCs, and (II) negative depletion incorporates common leukocyte antigen CD45-binding and granulocyte marker CD15 (fucosyltransferase 4  $\alpha$ 1,3 fucosyltransferase myeloid-specific; FUT4; SSEA-1)-targeting magnetic beads to bind white blood cells (WBCs).

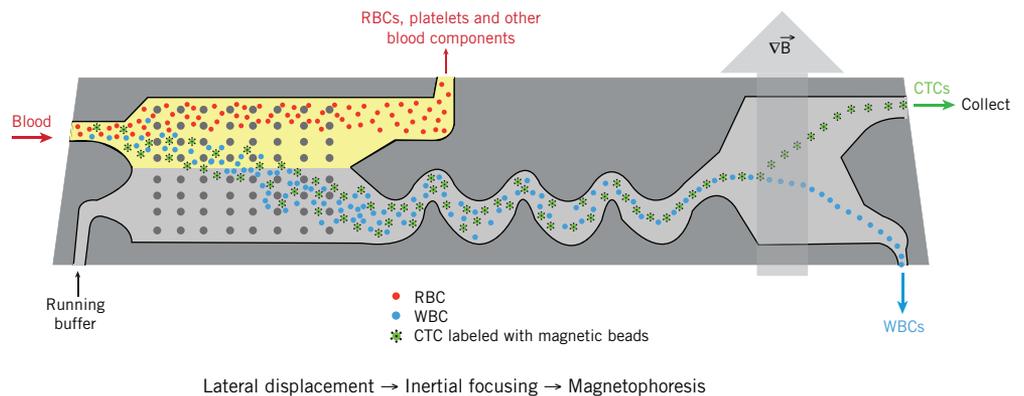
During lateral displacement, the sample moves through an array of microposts, spaced 32  $\mu$ m apart, which results in size-based separation and retention of nucleated cells. Red blood cells (RBCs), platelets and other components exit the device.

For inertial focusing, the nucleated cells enter an asymmetrical, curved channel and exit as a tight row of individual cells.

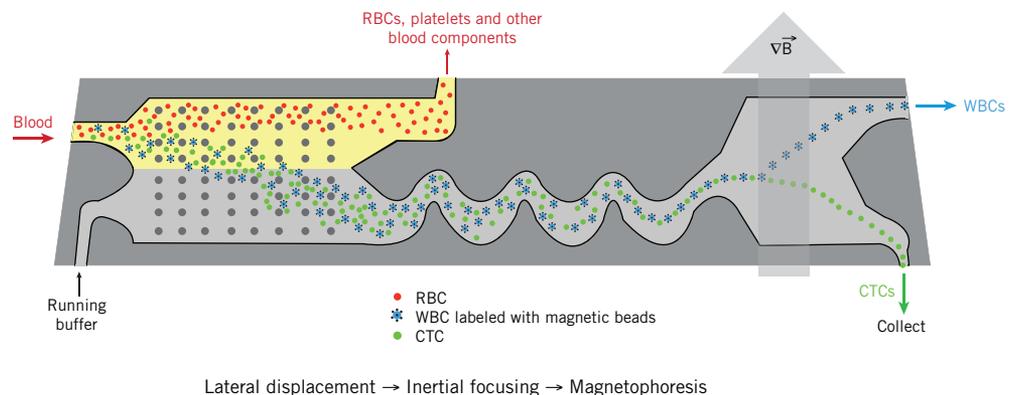
Finally, with magnetophoresis, a magnetic field ( $\nabla B$ ) separates magnetically labeled cells from unlabeled cells and provides an isolated supply of CTCs in solution.

In total, the device can process about 8 mL of whole blood per hour, translating to about 107 total cells per second. (Figure based on Figure 1a in ref. 1.)

## I Positive selection



## II Negative depletion



a significantly higher throughput and the ability to isolate CTCs without an immobilization step. The latter feature allowed greater capture numbers of CTCs in solution and opened up the potential for downstream molecular characterization of the CTCs.<sup>1</sup>

The team engineered two forms of the device. The <sup>pos</sup>CTC-iChip isolates EpCAM<sup>+</sup> CTCs, whereas the <sup>neg</sup>CTC-iChip isolates EpCAM<sup>-</sup> and EpCAM<sup>+</sup> CTCs (see Figure 1, “Isolating CTCs”). The first has the advantage of providing higher-purity populations of EpCAM<sup>+</sup> CTCs. The second provides a way to capture the elusive EpCAM<sup>-</sup> CTCs and has the advantage of isolating CTCs from any type of cancer without the need to rely on a known surface antigen to isolate CTCs. This feature opens up the possibility to use the CTC-iChip for virtually all cancers.

As proof of concept, the researchers spiked blood with one of five different cancer cell lines. The <sup>pos</sup>CTC-iChip isolated EpCAM<sup>+</sup> cancer cells but not cancer cells that had undergone EMT. The <sup>neg</sup>CTC-iChip isolated cancer cells that had undergone EMT as well as the parental cancer cells that had not.

In a head-to-head comparison, the <sup>neg</sup>CTC-iChip had an order of magnitude lower purification than the <sup>pos</sup>CTC-iChip.

The team compared the <sup>pos</sup>CTC-iChip with CellSearch using 42 blood samples from individuals with prostate, breast, pancreatic, colorectal or lung cancer. Both systems did equally well with 6 samples that contained >30 CTCs per 7.5 mL of blood. For the 36 samples with <30 CTCs per 7.5 mL, the number of CTCs isolated with the <sup>pos</sup>CTC-iChip was significantly higher in 22 cases ( $p < 0.001$ ). For the remaining 14 cases with <30 CTCs, neither system could adequately isolate cells.

CTCs isolated with the <sup>pos</sup>CTC-iChip were able to be molecularly characterized, as an RT-PCR assay correctly identified four positive samples from patients with cancer that had the EML4-ALK oncogenic fusion protein at their primary tumor.

The team then used the <sup>neg</sup>CTC-iChip to isolate CTCs from blood obtained from patients with metastatic breast cancer, metastatic pancreatic cancer or metastatic melanoma. The researchers also were able to use gene expression profiling to characterize single CTCs collected by the <sup>neg</sup>CTC-iChip from the blood of an individual with prostate cancer.

Results were published in *Science Translational Medicine*.

“Ongoing studies with the CTC-iChip are underway in the clinic to monitor tumor genotype and resistance to treatment in metastatic cancer for targeted therapies,” said corresponding author Mehmet Toner, professor of surgery at MGH and **Harvard Medical School**. “Our longer-term goal is early cancer detection.”

“On the technical side, we are working to make the CTC-iChip a one-piece integrated device,” Toner said.

Currently, the first step of size-based depletion of red blood cells and platelets is done on one chip. The second step of inertial focusing and deterministic magnetic sorting is done on a second chip.

“We are working to develop a device that includes the CTC isolation

features as well as components that will completely characterize the isolated cells,” said Robert McCormack, head of technology, innovation and strategy at Veridex. “Some possibilities are fluorescence *in situ* hybridization (FISH) to detect the presence or absence of specific DNA or RNA sequences, fluorescent antibodies to detect cell surface markers such as HER2 or estrogen receptor, or next-generation sequencing. We are presently in collaboration or setting up partnerships with molecular and diagnostic companies to make this happen.”

Toner added, “To increase purity with the <sup>neg</sup>CTC-iChip, we are developing better ways to label the white blood cells with magnetic beads for negative depletion. Most of the contaminating white blood cells in the product expressed *CD45* but somehow the beads didn’t attach to them. We are already an order-of-magnitude better than the published paper and comparable to <sup>pos</sup>CTC-iChip.”

#### Other options

Although J&J is one of the few companies developing next-generation CTC products, competition could come from other avenues for obtaining liquid biopsies that predict outcomes and potential for metastasis and therapeutic resistance. These include

circulating tumor DNA (ctDNA)<sup>5,6</sup> and, to a lesser extent, exosomes.<sup>7,8</sup>

In patients with cancer, 1%–10% of circulating or cell-free DNA derives from tumor cells,<sup>9</sup> so the challenge has been to design assays that are sufficiently sensitive to detect low levels of rare mutant ctDNA among the much higher background levels of wild-type ctDNA and total cell-free DNA.<sup>10</sup>

This April, a **Cancer Research UK Cambridge Institute** team used a method to isolate, amplify and sequence tumor-derived ctDNA fragments to monitor acquired resistance to cancer therapy.<sup>11</sup> The method identified mutations acquired over a one to two-year period that were associated with emergence of therapy resistance and concordant with mutations in two corresponding metastatic tumor biopsy samples.

The team next plans to work toward gaining clinical pathology accreditation in the U.K.

“ctDNA is simpler and likely cheaper. However, in the long run it is unlikely to capture all the complexity of the cancer for diagnosis and monitoring of all tumors,” said Toner. “There are many genetic lesions that can only be found at the RNA level and many characteristics of CTCs, such as epithelial-to-mesenchymal transition markers, that are expressed at the protein level.”

**Atlas Venture** partner Bruce Booth agreed. “ctDNA has the advantage of being far easier and cheaper to capture and analyze using new PCR-like techniques. It also could give insights into primary tumor genomic changes that might not be apparent in CTCs,” he said. “But intact CTCs have the advantage that they could help physicians characterize potential metastatic cells and their potential differences from the primary tumor, which could alter therapeutic choice. It’s not clear whether circulating DNA is from primary or metastatic tumors. Also, if CTCs could be captured and cultured, chemosensitivity testing

**“ctDNA has the advantage of being far easier and cheaper to capture and analyze using new PCR-like techniques. It also could give insights into primary tumor genomic changes that might not be apparent in CTCs. But intact CTCs have the advantage that they could help physicians characterize potential metastatic cells and their potential differences from the primary tumor, which could alter therapeutic choice.”**

**—Bruce Booth, Atlas Venture**

**“I think one important aspect that will need to be incorporated is sampling ctDNA or CTCs over long periods of time as patients respond to therapy. This will help us to identify longitudinal markers of disease progression, remission and/or relapse that will ultimately benefit the patient in terms of helping to select appropriate therapies.”**

—Paul Dempsey,  
Cynvenio Biosystems Inc.

funding to continue developing the technology.

“The closest thing to a head-to-head comparison so far between ctDNA and CTCs is whole-genome sequencing of the ctDNAs and enumeration of the CTCs,” said Paul Dempsey, CSO of **Cynvenio Biosystems Inc.** “With the new techniques, a head-to-head comparison to determine treatment responses could also include molecular characterization of CTCs, such as deep sequencing.”

Regardless of approach, “I think one important aspect that will need to be incorporated is sampling ctDNA or CTCs over long periods of time as patients respond to therapy,” said Dempsey. “This will help us to identify longitudinal markers of disease progression, remission and/or relapse that will ultimately benefit the patient in terms of helping to select appropriate therapies.”

Cynvenio offers its LiquidBiopsy lab service for recovering and analyzing CTCs.

“Right now, I think the field is still in the earliest innings of the game of ‘show me the data,’” Booth said.

A third approach for analyzing tumors involves exosome sequencing. Exosomes are small membrane vesicles, about 30–100 nm, that are shed by normal and cancerous cells. They carry diffusible factors, such as cytokines, growth factors and extracellular matrix molecules,

could be done to identify what therapeutics—or more likely, combination of therapeutics—are most likely to work against metastatic cancer cells in those patients.”

Atlas had invested in On-Q-ity, which was developing a microfluidic chip that captured CTCs based on affinity and cell size. Each chip used several hundred thousand micro-sized posts covered with antibodies to capture the CTCs. **But the company failed to attract sufficient**

and mediate local and systemic transfer of mRNAs, microRNAs and proteins.<sup>8,12</sup>

These exosome-packaged molecules might also provide cancer biomarkers and could do so with less degradation of the RNA within the exosomes than RNA that is freely circulating in the blood.

**Exosome Diagnostics Inc.** has started clinical trials of its tests in colon cancer, melanoma and glioblastoma multiforme (GBM).

**NX PharmaGen** offers NeXosome, a prenatal and oncology diagnostics proteomics platform that permits analysis of protein biomarkers shed specifically from placental or tumor cells within protected exosomes.

Multiple patents covering various aspects of the technology are at different stages of filing and are exclusively licensed to J&J.

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**Cynvenio Biosystems Inc.**, Westlake Village, Calif.  
**Exosome Diagnostics Inc.**, New York, N.Y.  
**Harvard Medical School**, Cambridge, Mass.  
**Johnson & Johnson** (NYSE:JNJ), New Brunswick, N.J.  
**Massachusetts General Hospital**, Boston, Mass.  
**NX PharmaGen**, Louisville, Ky.

## 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
<b>Cancer</b>				
Brain cancer	Fibroblast growth factor receptor 1 (FGFR1; CD331)	<p>Studies in mice and in patient samples suggest inhibiting FGFR1 could be useful for treating a subset of pediatric gliomas that have genomic alterations in the target. Whole-genome sequencing identified duplications within <i>FGFR1</i> in 2 of 39 pediatric gliomas. In mice, transplantation of astrocytes expressing the altered form of <i>FGFR1</i> led to tumor development, whereas transplantation of astrocytes expressing wild-type <i>FGFR1</i> did not. Next steps could include testing the effects of FGFR1 inhibitors in mouse models for glioma with the alteration.</p> <p>At least six selective FGFR1 inhibitors are in Phase II testing or earlier to treat various cancers.</p> <p><b>SciBX 6(17); doi:10.1038/scibx.2013.409</b> <b>Published online May 2, 2013</b></p>	Patent and licensing status unavailable	<p>Zhang, J. <i>et al. Nat. Genet.</i>; published online April 14, 2013; doi:10.1038/ng.2611</p> <p><b>Contact:</b> David W. Ellison, St. Jude Children's Research Hospital, Memphis, Tenn. e-mail: <a href="mailto:david.ellison@stjude.org">david.ellison@stjude.org</a></p>
Breast cancer	<i>Dedicator of cytokinesis 1</i> ( <i>DOCK1</i> )	<p>Patient sample and mouse studies suggest inhibiting <i>DOCK1</i> could help prevent breast cancer metastasis. In tumor samples from patients with breast cancer, increased expression of <i>DOCK1</i> was associated with poor survival in HER2 (EGFR2; ERBB2; neu)-positive and basal breast cancers. In a mouse model for HER2-positive breast cancer, mammary gland-specific knockout of <i>Dock1</i> decreased primary tumor burden and metastasis to lungs compared with no knockout. In mice injected with HER2-positive breast cancer, small interfering RNA-mediated knockdown of <i>DOCK1</i> led to decreased metastases compared with no knockdown. Next steps include identifying compounds that block <i>DOCK1</i> and evaluating them in animal models for cancer.</p> <p><b>SciBX 6(17); doi:10.1038/scibx.2013.410</b> <b>Published online May 2, 2013</b></p>	Patent application filed; available for licensing from Kyushu University's Intellectual Property Management Center	<p>Laurin, M. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online April 16, 2013; doi:10.1073/pnas.1213050110</p> <p><b>Contact:</b> Jean-François Côté, Montreal Institute of Clinical Research, Montreal, Quebec, Canada e-mail: <a href="mailto:jean-francois.cote@ircm.qc.ca">jean-francois.cote@ircm.qc.ca</a></p>
Cancer	Fibroblast growth factor receptor (FGFR)	<p>Two separate studies identified an allosteric inhibitor of FGFR that could help treat cancer. In the first study, NMR, molecular dynamics and X-ray crystallography analysis found that the small molecule SSR128129E (SSR) bound the extracellular D3 domain at a site distinct from FGFR ligand binding and acted as an allosteric inhibitor by altering FGF binding site conformation. In the second study, oral dosing of SSR decreased tumor size, tumor invasiveness and metastasis in mouse models for cancer compared with oral dosing of vehicle. In mouse models for pancreatic and colorectal cancers, SSR plus a VEGF receptor 2 (KDR/Flk-1; VEGFR-2) inhibitor decreased tumor growth better than either treatment alone. Next steps include clinical testing of SSR analogs. At least six selective FGFR inhibitors are in Phase II testing or earlier to treat various cancers (<i>see FGFR allosteric inhibition, page 4</i>).</p> <p><b>SciBX 6(17); doi:10.1038/scibx.2013.411</b> <b>Published online May 2, 2013</b></p>	SSR and related compounds from both studies patented by Sanofi; Sanofi and the Flanders Institute for Biotechnology (VIB) have a joint patent covering methods used; unavailable for licensing	<p>Herbert, C. <i>et al. Cancer Cell</i>; published online April 15, 2013; doi:10.1016/j.ccr.2013.02.018</p> <p><b>Contact:</b> Françoise Bono, Sanofi Research and Development, Toulouse, France e-mail: <a href="mailto:francoise.bono@sanofi-aventis.com">francoise.bono@sanofi-aventis.com</a></p> <p><b>Contact:</b> Harald Schwalbe, Goethe University Frankfurt, Frankfurt, Germany e-mail: <a href="mailto:schwalbe@nmr.uni-frankfurt.de">schwalbe@nmr.uni-frankfurt.de</a></p> <p><b>Contact:</b> Francesco Luigi Gervasio, Spanish National Cancer Research Centre (CNIO), Madrid, Spain e-mail: <a href="mailto:fgervasio@cnio.es">fgervasio@cnio.es</a></p> <p>Bono, F. <i>et al. Cancer Cell</i>; published online April 15, 2013; doi:10.1016/j.ccr.2013.02.019</p> <p><b>Contact:</b> Peter Carmeliet, Catholic University Leuven, Leuven, Belgium e-mail: <a href="mailto:peter.carmeliet@vib-kuleuven.be">peter.carmeliet@vib-kuleuven.be</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Liver cancer	Calcium channel voltage-dependent $\alpha 2/\delta$ subunit 1 (CACNA2D1)	<p>Mouse and <i>in vitro</i> studies suggest targeting CACNA2D1 could help treat hepatocellular carcinoma (HCC). In human HCC cell lines, the mAb 1B50-1, which binds to CACNA2D1 isoform 5, selectively targeted and killed populations of tumor-initiating cells. In mouse xenograft models for HCC, 1B50-1 decreased tumor growth and the subpopulation of tumor-initiating cells compared with an IgG control. Next steps could include testing the antibody in additional models for HCC.</p> <p><b>SciBX 6(17); doi:10.1038/scibx.2013.412</b>  <b>Published online May 2, 2013</b></p>	Patent and licensing status unavailable	<p>Zhao, W. <i>et al. Cancer Cell</i>; published online April 15, 2013; doi:10.1016/j.ccr.2013.02.025  <b>Contact:</b> Zhiqian Zhang, Peking University Cancer Hospital and Institute, Beijing, China  e-mail: <a href="mailto:zlzqzhang@bjmu.edu.cn">zlzqzhang@bjmu.edu.cn</a>  <b>Contact:</b> Baocai Xing, same affiliation as above  e-mail: <a href="mailto:xingbaocai88@sina.com">xingbaocai88@sina.com</a></p>
Pancreatic cancer	Not applicable	<p>Mouse studies suggest radionuclide-labeled <i>Listeria monocytogenes</i> could help treat metastatic pancreatic cancer. In a mouse model for metastatic pancreatic cancer, an attenuated strain of <i>L. monocytogenes</i> showed greater infection and replication in metastatic lesions than in primary tumors and normal tissues. In the mouse model, attenuated <i>L. monocytogenes</i> linked to rhenium-188 (<math>^{188}\text{Re}</math>) decreased the number and size of metastatic lesions compared with the bacterium or <math>^{188}\text{Re}</math> alone. Next steps include testing the <math>^{188}\text{Re}</math>-labeled <i>L. monocytogenes</i> in xenograft models for pancreatic cancer.</p> <p>Advaxis Inc.'s Lovaxis C (ADXS11-001), a live <i>L. monocytogenes</i>-based immunotherapy expressing HPV type 16 E7, is in Phase II testing to treat cervical cancer and Phase I/II testing to treat colorectal and head and neck cancers.</p> <p>Aduro BioTech Inc.'s CRS-207 (ANZ-207), a live, attenuated strain of <i>L. monocytogenes</i> that expresses human mesothelin, is in Phase II testing to treat pancreatic cancer and Phase I testing to treat mesothelioma.</p> <p>(See <b>Add radioactivity to the <i>Listeria</i></b>, page 6).</p> <p><b>SciBX 6(17); doi:10.1038/scibx.2013.413</b>  <b>Published online May 2, 2013</b></p>	Patented by the Albert Einstein College of Medicine of Yeshiva University; available for licensing	<p>Quispe-Tintaya, W. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online April 22, 2013; doi:10.1073/pnas.1211287110  <b>Contact:</b> Claudia Gravekamp, Albert Einstein College of Medicine of Yeshiva University, Bronx, N.Y.  e-mail: <a href="mailto:claudia.gravekamp@einstein.yu.edu">claudia.gravekamp@einstein.yu.edu</a>  <b>Contact:</b> Ekaterina Dadachova, same affiliation as above  e-mail: <a href="mailto:ekaterina.dadachova@einstein.yu.edu">ekaterina.dadachova@einstein.yu.edu</a></p>
<b>Endocrine/metabolic disease</b>				
Diabetes	Chemokine CX3C motif ligand 1 (CX3CL1; fractalkine); chemokine CX3C motif receptor 1 (CX3CR1)	<p><i>In vitro</i> and mouse studies suggest fractalkine or agonists of its receptor, CX3CR1, could help treat diabetes. In mice, an anti-fractalkine antibody or knockout of <i>Cx3cr1</i> decreased glucose tolerance and glucose-stimulated insulin secretion compared with vehicle or no knockout, respectively. In mice, soluble fractalkine increased glucose tolerance and insulin secretion compared with what was seen in <i>Cx3cr1</i> knockouts. In human islets, fractalkine increased glucose-stimulated insulin secretion compared with no treatment. Next steps could include testing soluble fractalkine in mouse models for diabetes.</p> <p><b>SciBX 6(17); doi:10.1038/scibx.2013.414</b>  <b>Published online May 2, 2013</b></p>	Patent and licensing status unavailable	<p>Lee, Y.S. <i>et al. Cell</i>; published online April 11, 2013; doi:10.1016/j.cell.2013.03.001  <b>Contact:</b> Jerrold Olefsky, University of California, San Diego, La Jolla, Calif.  e-mail: <a href="mailto:jolefsky@ucsd.edu">jolefsky@ucsd.edu</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Hypercholesterolemia; hyperlipidemia; dyslipidemia	SEC24 family member A (SEC24A); proprotein convertase subtilisin/kexin type 9 (PCSK9); low-density lipoprotein receptor (LDLR)	<p>Mouse studies suggest inhibiting SEC24A could help treat hypercholesterolemia and related metabolic indications. In mice, a Sec24a deficiency decreased hepatic secretion of Pcsk9 and increased Ldlr expression compared with no Sec24a deficiency. In the Sec24a-deficient mice, circulating levels of high-density lipoprotein, low-density lipoprotein and total cholesterol were lower than those in nondeficient controls. Next steps include testing the effects of SEC24A knockdown in human hepatocytes.</p> <p>AMG 145, a human mAb against PCSK9 from Amgen Inc., is in Phase III testing to treat hypercholesterolemia, hyperlipidemia and dyslipidemia.</p> <p>REGN727 (SAR236553), a human mAb targeting PCSK9 from Regeneron Pharmaceuticals Inc. and Sanofi, is in Phase III testing to treat hypercholesterolemia.</p> <p>PF-04950615 (PF-4950615; RN315), a PCSK9 inhibitor from Pfizer Inc., is in Phase II testing to treat hypercholesterolemia and acute coronary syndrome.</p> <p><b>SciBX 6(17); doi:10.1038/scibx.2013.415</b> Published online May 2, 2013</p>	<p>Unpatented; available for partnering or licensing from the University of Michigan</p> <p>Contact: Robin Razor, University of Michigan, Ann Arbor, Mich. e-mail: <a href="mailto:robinlr@umich.edu">robinlr@umich.edu</a></p>	<p>Chen, X.-W. <i>et al. eLife</i>; published online April 9, 2013; doi:10.7554/eLife.00444</p> <p>Contact: David Ginsburg, University of Michigan, Ann Arbor, Mich. e-mail: <a href="mailto:ginsburg@umich.edu">ginsburg@umich.edu</a></p>
<b>Hepatic disease</b>				
Liver failure	Mitogen- activated protein kinase kinase 4 (MAP2K4; MKK4)	<p>Mouse studies suggest antagonizing MKK4 could help treat liver failure. In mouse models for acute or chronic liver failure, small interfering RNA-mediated knockdown of <i>Mkk4</i> decreased fibrosis and increased both hepatocyte regeneration and survival compared with no knockdown. Next steps include reproducing the results in cultured human hepatocytes and identifying selective MKK4 inhibitors.</p> <p><b>SciBX 6(17); doi:10.1038/scibx.2013.416</b> Published online May 2, 2013</p>	<p>Patents pending; available for licensing</p>	<p>Wuestefeld, T. <i>et al. Cell</i>; published online April 11, 2013; doi:10.1016/j.cell.2013.03.026</p> <p>Contact: Lars Zender, Hannover Medical School, Hannover, Germany e-mail: <a href="mailto:lars.zender@med.uni-tuebingen.de">lars.zender@med.uni-tuebingen.de</a></p>
<b>Infectious disease</b>				
HCV; HIV/AIDS	Type I interferon receptor	<p>Mouse studies suggest neutralizing interferon (IFN) receptor signaling could help treat persistent HIV or HCV infections. In mice infected with a persistent strain of lymphocytic choriomeningitis virus (LCMV), expression of IFN, proinflammatory cytokines and immunosuppressive factors was greater than that in mice infected with an acute LCMV strain. In mice with persistent LCMV infection, prophylactic or therapeutic treatment with an anti-IFN receptor antibody initially increased viral loads but decreased or eliminated the virus during the chronic phase of infection compared with isotype control treatment. Next steps include testing the strategy in human viral infections, including HIV.</p> <p><b>SciBX 6(17); doi:10.1038/scibx.2013.417</b> Published online May 2, 2013</p>	<p>Patent and licensing status unavailable for findings from first study</p> <p>Provisional patent application filed for findings from second study; available for licensing</p>	<p>Teijaro, J.R. <i>et al. Science</i>; published online April 12, 2013; doi:10.1126/science.1235214</p> <p>Contact: Michael B.A. Oldstone, The Scripps Research Institute, La Jolla, Calif. e-mail: <a href="mailto:mboabo@scripps.edu">mboabo@scripps.edu</a></p> <p>Wilson, E.B. <i>et al. Science</i>; published online April 12, 2013; doi:10.1126/science.1235208</p> <p>Contact: David G. Brooks, University of California Los Angeles, Calif. e-mail: <a href="mailto:dbrooks@microbio.ucla.edu">dbrooks@microbio.ucla.edu</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
<b>Neurology</b>				
Alzheimer's disease (AD)	AMP-activated protein kinase (AMPK); $\beta$ -amyloid 42 (A $\beta$ 42); calcium calmodulin-dependent protein kinase 2 (CAMKK2)	Mouse and cell culture studies suggest inhibiting the CAMKK2/AMPK pathway could help prevent A $\beta$ 42 toxicity in AD. In cultured mouse hippocampal neurons, knockout or pharmacological inhibition of Camkk2 decreased A $\beta$ 42 oligomer-induced synaptotoxicity compared with no knockout or inhibition. In cell culture studies in neurons, the A $\beta$ 42 oligomers induced AMPK-mediated phosphorylation of microtubule-associated protein- $\tau$ (MAPT; TAU; FTDP-17). In mice overexpressing A $\beta$ 42 in the hippocampus, inhibiting Camkk2/Ampk signaling decreased the loss of dendritic spines compared with no inhibition. Next steps include validating the long-term protective effects of blocking CAMKK2/AMPK signaling on additional measures of AD histopathology.  <b>SciBX 6(17); doi:10.1038/scibx.2013.418</b> <b>Published online May 2, 2013</b>	Unpatented; licensing status not applicable	Mairet-Coello, G. <i>et al. Neuron</i> ; published online April 10, 2013; doi:10.1016/j.neuron.2013.02.003 <b>Contact:</b> Franck Polleux, The Scripps Research Institute, La Jolla, Calif. e-mail: <a href="mailto:polleux@scripps.edu">polleux@scripps.edu</a>
Pain	Fatty acid amide hydrolase (FAAH)	Mouse and <i>in vitro</i> studies identified $\alpha$ -ketoazole-based covalent inhibitors of FAAH that could be useful for treating pain. <i>In vitro</i> , the lead inhibitor caused irreversible inhibition of FAAH by covalently binding to serine 241 and cysteine 269 in the enzyme's active site. In a mouse model for neuropathic pain, the lead inhibitor lowered allodynia with a longer duration of effect than a reversible FAAH inhibitor. Next steps include evaluating the compounds in additional animal models for pain. Vernalis plc's V158866, a small molecule FAAH inhibitor, is in Phase II trials to treat pain.  <b>SciBX 6(17); doi:10.1038/scibx.2013.419</b> <b>Published online May 2, 2013</b>	Unpatented; compounds available for licensing	Otrubova, K. <i>et al. J. Am. Chem. Soc.</i> ; published online April 12, 2013; doi:10.1021/ja4014997 <b>Contact:</b> Dale L. Boger, The Scripps Research Institute, La Jolla, Calif. e-mail: <a href="mailto:boger@scripps.edu">boger@scripps.edu</a>
<b>Various</b>				
Coronary artery disease (CAD); Parkinson's disease (PD)	Myeloperoxidase (MPO)	<i>In vitro</i> and rat studies suggest a series of MPO inhibitors could help treat inflammation-associated diseases such as CAD and PD. SAR studies and <i>in vitro</i> testing of alkylindoles identified compounds that selectively inhibit MPO with low nanomolar IC <sub>50</sub> values. In a low-density lipoprotein (LDL) oxidation assay, two of the compounds inhibited MPO-mediated LDL oxidation with low nanomolar IC <sub>50</sub> values. Select compounds showed a good safety profile in normal rats. Next steps could include testing the compounds in animal models for CAD. AZD3241, an MPO inhibitor from AstraZeneca plc, is in Phase II testing to treat PD.  <b>SciBX 6(17); doi:10.1038/scibx.2013.420</b> <b>Published online May 2, 2013</b>	Patent and licensing status unavailable	Soubhye, J. <i>et al. J. Med. Chem.</i> ; published online April 14, 2013; doi:10.1021/jm4001538 <b>Contact:</b> Pierre G. Van Antwerpen, Free University of Brussels, Brussels, Belgium e-mail: <a href="mailto:pvantwer@ulb.ac.be">pvantwer@ulb.ac.be</a>

## 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>			
Cell-free, circulating tumor DNA to monitor acquired resistance to cancer therapy	A method to isolate, amplify and sequence tumor-derived, circulating DNA fragments could help monitor acquired resistance to cancer therapy without requiring a biopsy. A high-throughput, deep-sequencing platform was adapted to amplify and sequence DNA fragments in blood samples from two patients with breast cancer, three patients with ovarian cancer and one patient with lung cancer. The platform identified mutations acquired over a one to two-year period that were associated with emergence of therapy resistance and concordant with mutations in two corresponding metastatic tumor biopsy samples. Next steps include working toward gaining clinical pathology accreditation ( <i>see CTCs gain character(ization)</i> , page 8).	Unpatented; licensing status not applicable	Murtaza, M. <i>et al. Nature</i> ; published online April 7, 2013; doi:10.1038/nature12065 <b>Contact:</b> Nitzan Rosenfeld, Cancer Research UK Cambridge Institute and University of Cambridge, Cambridge, U.K. e-mail: <a href="mailto:nitzan.rosenfeld@cruk.cam.ac.uk">nitzan.rosenfeld@cruk.cam.ac.uk</a>
	<b>SciBX 6(17); doi:10.1038/scibx.2013.421</b> Published online May 2, 2013		
High throughput, microfluidic platform for the isolation of epithelial cell adhesion molecule (EpCAM)-positive and EpCAM-negative circulating tumor cells (CTCs) from blood samples	A high throughput, microfluidic platform called the CTC-iChip could help guide cancer diagnosis and prognosis. The platform separates CTCs from the blood via a series of steps involving microfluidic methods for rare cell handling and magnetic-based cell sorting. The CTC-iChip was used to isolate EpCAM-positive CTCs from the blood of patients with prostate, lung, breast, pancreatic or colorectal cancer and EpCAM-negative CTCs from the blood of patients with metastatic breast cancer, pancreatic cancer or melanoma. The CTCs were of sufficient quality that they could be characterized using immunohistochemistry, RNA molecular analysis or gene expression profiling. Next steps include adding molecular assay components to the device ( <i>see CTCs gain character(ization)</i> , page 8).	Multiple patents covering various aspects of the technology at different stages of filing; exclusively licensed to Johnson & Johnson	Ozkumur, E. <i>et al. Sci. Transl. Med.</i> ; published online April 3, 2013; doi:10.1126/scitranslmed.3005616 <b>Contact:</b> Mehmet Toner, Harvard Medical School, Boston, Mass. e-mail: <a href="mailto:mtoner@hms.harvard.edu">mtoner@hms.harvard.edu</a>
	<b>SciBX 6(17); doi:10.1038/scibx.2013.422</b> Published online May 2, 2013		
<b>Computational models</b>			
Algorithm for chemical space exploration with stochastic search (ACSESS) to develop structurally diverse chemical compound libraries for drug screening	ACSESS could help create diverse chemical libraries for drug screening. ACSESS can be used to design libraries with specific chemical diversity or decrease library size by selecting a representative set of the library's diversity. As proof of concept, ACSESS identified a library representative of the full small molecule universe using filters for size, reactivity, stability and drug-like properties that was more structurally complete than existing libraries. ACSESS also was used to design a reduced library of compounds that have 13 or fewer heavy atoms that had comparable diversity to the complete library. Next steps include designing libraries for general screening and specific targets.	Unpatented; software available for licensing from OpenEye Scientific Software Inc. and Duke University for commercial use; free licenses available for noncommercial use	Virshup, A.M. <i>et al. J. Am. Chem. Soc.</i> ; published online April 2, 2013; doi:10.1021/ja401184g <b>Contact:</b> David N. Beratan, Duke University, Durham, N.C. e-mail: <a href="mailto:david.beratan@duke.edu">david.beratan@duke.edu</a> <b>Contact:</b> Weitao Yang, same affiliation as above e-mail: <a href="mailto:weitao.yang@duke.edu">weitao.yang@duke.edu</a>
	<b>SciBX 6(17); doi:10.1038/scibx.2013.423</b> Published online May 2, 2013		
<b>Disease models</b>			
Frog model for scoliosis	A frog model for scoliosis caused by ear damage could be useful for studying the pathophysiology of the disease. Inner ear abnormalities have previously been linked to idiopathic cases of scoliosis. In this model, tadpoles were subjected to unilateral removal of labyrinthine end organs and then raised into adult frogs. The tadpoles showed degeneration of vestibular afferents and loss of vestibulospinal neurons. The frogs that developed from these tadpoles showed postural asymmetry and skeletal distortions similar to those observed in patients with scoliosis. Next steps could include using the model to identify molecules that prevent development of disease pathology.	Patent and licensing status unavailable	Lambert, F.M. <i>et al. J. Neurosci.</i> ; published online April 17, 2013; doi:10.1523/JNEUROSCI.4842-12.2013 <b>Contact:</b> Hans Straka, Ludwig Maximilian University of Munich, Munich, Germany e-mail: <a href="mailto:straka@lmu.de">straka@lmu.de</a>
	<b>SciBX 6(17); doi:10.1038/scibx.2013.424</b> Published online May 2, 2013		

## This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
<b>Drug platforms</b>			
Chimeric antigen receptor (CAR)-expressing T cells lacking negative regulators of T cell receptor (TCR) signaling	CAR-expressing T cells that lack negative regulators of TCR signaling could be useful as antitumor T cell therapies. Diacylglycerol kinases (DGKs) are known negative regulators of TCR signaling. In a xenograft mouse model for established human mesothelioma, CAR-expressing murine T cells that lacked DGKs significantly decreased tumor growth compared with cells that expressed DGKs ( $p < 0.05$ ). Next steps include developing human CAR-expressing T cells that also express DGK-targeting small interfering RNAs or dominant negative forms of DGKs.  <b>SciBX 6(17); doi:10.1038/scibx.2013.425</b> <b>Published online May 2, 2013</b>	Patent application filed; available for licensing through the University of Pennsylvania's Center for Technology Transfer	Riese, M.J. <i>et al. Cancer Res.</i> ; published online April 10, 2013; doi:10.1158/0008-5472.CAN-12-3874 <b>Contact:</b> Steven M. Albelda, University of Pennsylvania, Philadelphia, Pa. e-mail: <a href="mailto:albelda@mail.med.upenn.edu">albelda@mail.med.upenn.edu</a>
Fibroblast-derived induced oligodendrocyte progenitor cells (iOPCs) to treat myelination disorders	Two separate studies developed methods to reprogram mouse fibroblasts into iOPCs and suggested they could be used to help treat diseases with myelination dysfunction such as multiple sclerosis (MS). In the first study, transduction of the transcription factors SRY-box containing gene 10 (Sox10), oligodendrocyte transcription factor 2 (Olig2) and NK6 homeobox 2 (Nkx6-2) in mouse fibroblasts generated iOPCs that could be differentiated into oligodendrocytes. Coculturing brain slices from mice lacking myelin basic protein (Mbp) with the iOPCs caused axon myelination. In mice lacking Mbp, spinal cord injection of iOPCs led to axon myelination. In the second study, transduction of mouse or rat fibroblasts with the transcription factors Sox10, Olig2 and zinc finger protein 536 (Zfp536) also generated iOPCs that could be differentiated into oligodendrocytes. Transplantation of iOPCs to the brain led to axon myelination in Mbp-deficient mice. Next steps include using the technology on human cells to generate patient-specific iOPCs and improving the reprogramming process to increase the number of induced cells.  <b>SciBX 6(17); doi:10.1038/scibx.2013.426</b> <b>Published online May 2, 2013</b>	Patent application filed by the Myelin Repair Foundation and assigned to Case Western Reserve University for findings in first study; available for licensing  Patent application filed for findings in second study; licensing status unavailable	Najm, F.J. <i>et al. Nat. Biotechnol.</i> ; published online April 14, 2013; doi:10.1038/nbt.2561 <b>Contact:</b> Paul J. Tesar, Case Western Reserve University School of Medicine, Cleveland, Ohio e-mail: <a href="mailto:paul.tesar@case.edu">paul.tesar@case.edu</a>  Yang, N. <i>et al. Nat. Biotechnol.</i> ; published online April 14, 2013; doi:10.1038/nbt.2564 <b>Contact:</b> Marius Wernig, Stanford University School of Medicine, Stanford, Calif. e-mail: <a href="mailto:wernig@stanford.edu">wernig@stanford.edu</a>
<b>Imaging</b>			
Functional MRI (fMRI) to monitor psychosis	Mouse and human studies suggest fMRI could be useful for detecting the onset of psychosis. In patients with a history of psychosis, fMRI at the onset of psychotic episodes showed decreased hippocampus size but increased hippocampal activity compared with that of nonpsychotic controls. In an fMRI study of mice with acute ketamine-induced psychosis, imaging showed that LY379286, a metabotropic glutamate receptor subtype 2 (mGluR2; GRM2) and mGluR3 (GRM3) agonist, decreased hippocampal atrophy and hyperactivity compared with vehicle. Next steps include scaling up clinical imaging studies to identify at-risk patients and testing the effect of mGluR2 and/or mGluR3 modulators on hippocampal activity. Eli Lilly and Co.'s Pomaglumetad methionil, an oral prodrug of an mGluR2 and mGluR3 agonist, was discontinued last year after missing the endpoints in a Phase III trial to treat schizophrenia. Roche, Addex Therapeutics Ltd. and Taisho Pharmaceutical Co. Ltd. have compounds in this class in Phase I and II testing to treat various neuropsychiatric indications.  <b>SciBX 6(17); doi:10.1038/scibx.2013.427</b> <b>Published online May 2, 2013</b>	Unpatented; licensing status not applicable	Schobel, S.A. <i>et al. Neuron</i> ; published online April 10, 2013; doi:10.1016/j.neuron.2013.02.011 <b>Contact:</b> Scott A. Small, Columbia University Medical Center, New York, N.Y. e-mail: <a href="mailto:sas68@columbia.edu">sas68@columbia.edu</a> <b>Contact:</b> Holly Moore, same affiliation as above e-mail: <a href="mailto:hm2035@columbia.edu">hm2035@columbia.edu</a>

## This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
<b>Markers</b>			
Brown adipose tissue (BAT) in adult humans	<p>Two separate studies in humans identified physiological and genetic markers of BAT, which could aid the development of BAT-targeted therapies. Prior studies had challenged whether humans possess BAT. Postmortem MRI and histopathology studies of human infants identified a depot of cells with markers comparable to those of BAT cells in animals. In biopsies from adult humans, cells with histological and genetic markers of BAT were found in the back of the neck. Next steps include developing strategies to induce proliferation in existing BAT cells or transdifferentiation of other cells into BAT cells.</p> <p>Ember Therapeutics Inc. has discovery-stage compounds that induce BAT development to treat obesity and metabolic syndrome.</p> <p><b>SciBX 6(17); doi:10.1038/scibx.2013.428</b>  <b>Published online May 2, 2013</b></p>	Findings for both studies unpatented; licensing status not applicable	<p>Cypess, A.M. <i>et al. Nat. Med.</i>; published online April 21, 2013; doi:10.1038/nm.3112  <b>Contact:</b> Aaron M. Cypess, Harvard Medical School, Boston, Mass.  e-mail:  <a href="mailto:aaron.cypess@joslin.harvard.edu">aaron.cypess@joslin.harvard.edu</a></p> <p>Lidell, M.E. <i>et al. Nat. Med.</i>; published online April 21, 2013; doi:10.1038/nm.3017  <b>Contact:</b> Sven Enerbäck, University of Gothenburg, Gothenburg, Sweden  e-mail:  <a href="mailto:sven.enerback@medgen.gu.se">sven.enerback@medgen.gu.se</a></p>

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