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Blood brain barrier in a dish

By Lev Osherovich, Senior Writer

Wisconsin researchers have used cultured human stem cells to engineer an artificial blood brain barrier.¹ The technology could help companies identify drug candidates that readily enter the brain or that decrease blood brain barrier permeability.

The blood brain barrier (BBB) is a system of transporters and physical barriers in the endothelial cells of the brain vasculature that keeps most foreign molecules out of the brain. Breakdown of the BBB contributes to inflammatory and autoimmune diseases such as multiple sclerosis (MS) as well as certain forms of epilepsy.

Because the BBB arises from the complex interplay between endothelial cells, neurons and glia, it has proven difficult to recapitulate *in vitro*. Thus, testing for BBB-modulating drugs has been a slow, hit-or-miss process that requires testing in animals.

Previous *in vitro* systems for studying the BBB have suffered from numerous shortcomings, including poor scalability and inadequate separation of blood and brain compartments. Now, a team led by Eric Shusta, associate professor of chemical and biological engineering at the **University of Wisconsin-Madison**, has overcome these problems by building an *in vitro* BBB from scratch using pluripotent stem cells as the starting point.

Shusta's team worked out a protocol to coax the stem cells into developing into an endothelial cell layer with BBB-like properties, including drug-pumping surface proteins and tight junctions between the cells (see Figure 1, "Building a better blood brain barrier").

"People have previously used primary cell culture, but this can't be scaled to the point of being useful for screening," said Shusta. "When you take cells out of a mature brain and put them in cell culture out of their context, you lose a lot of the properties of the BBB. Instead, we're growing cells from the start to develop these properties."

Culture club

Shusta's team set out to grow human pluripotent stem cells in an environment that mimics that of the developing brain. The researchers cultured human pluripotent stem cell lines in medium that had been exposed to cultured endothelial cells. As a result, the medium was laden with growth factors that encouraged endothelial cell development.

Indeed, a subset of cells grown in the endothelial cell-conditioned medium had higher levels of BBB-associated markers such as tight junction proteins and glucose transporters than cells grown in unconditioned medium.



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Although some stem cells differentiated into endothelial cell precursors, other cells in the culture developed neuron-like characteristics and produced peptides including wingless-type MMTV integration site (WNT) family members that in turn promoted further development of the endothelial cells.

When purified and cultured on their own, the endothelial cell precursors from this mixed culture formed a layer of cells that resembled the endothelial layer of brain vascular tissue.

Next, Shusta's team set up an assay of BBB function in which the endothelial cells were cocultured with brain-derived astrocytes, with the two cell types separated by a porous filter. In this arrangement, the astrocytes provide soluble factors that direct the endothelial cells to form a tightly associated layer with BBB-like properties.

Indeed, the artificial BBB made by Shusta's cells was markedly stronger than other *in vitro* BBB models. The cell layer had a high electrical resistance, indicating the tight junctions between the cells blocked the flow of ions and other soluble molecules from one side of the layer to the other.

"Our electrical resistance is substantially higher than any other systems, 50–100 times better than other human or animal models," said Shusta. "This is a measure of how tight the junctions are."

In an assay of small molecule transport, Shusta's artificial BBB excluded compounds known to be blocked from the brain and admitted

"When you take cells out of a mature brain and put them in cell culture out of their context, you lose a lot of the properties of the BBB. Instead, we're growing cells from the start to develop these properties."

**—Eric Shusta,
University of Wisconsin–Madison**

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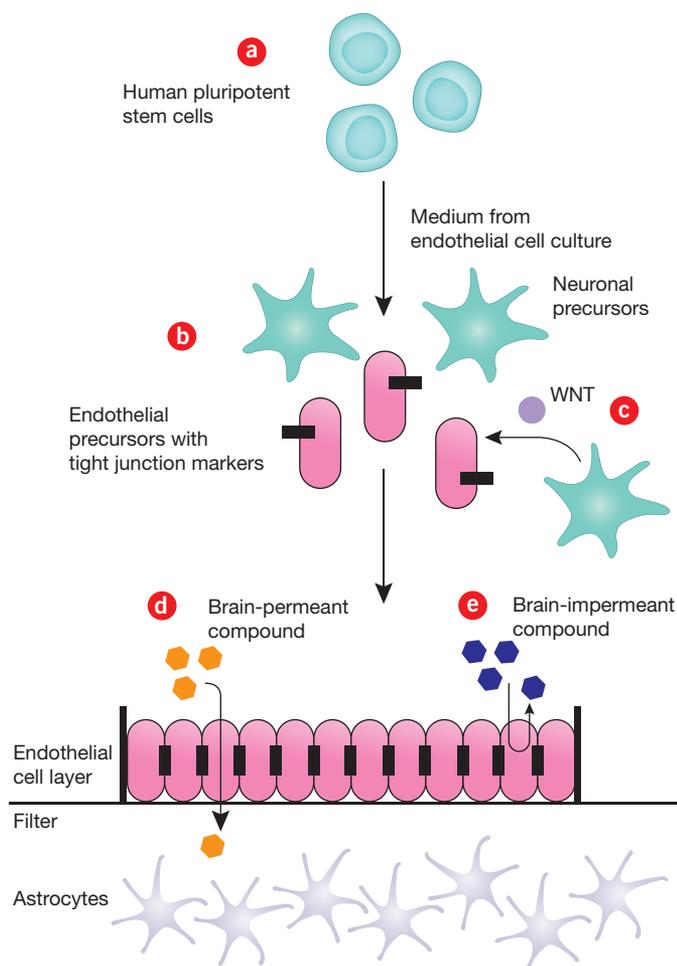


Figure 1. Building a better blood brain barrier. Lippmann *et al.* have modeled the blood brain barrier (BBB) *in vitro* using human pluripotent stem cells.

The technique involves treating undifferentiated human pluripotent stem cells [a] with cell culture medium that has previously been used to grow endothelial cells. The stem cells differentiate into a mixed population of neuronal and endothelial cell precursors [b] that express tight junction markers. The neurons in the mixed culture secrete developmental factors including wingless-type MMTV integration site (WNT) family members that guide the differentiation of endothelial cells [c]. When cultured with rat astrocytes in an *in vitro* BBB assay, the resulting endothelial cells form tight junctions and show BBB activity, including the transport of brain-permeant compounds [d] and the exclusion of brain-impermeant compounds [e].

molecules such as glucose that can readily cross the BBB.

Shusta has filed to patent the technique. The patents are available for licensing from the **Wisconsin Alumni Research Foundation**, the technology transfer office of the University of Wisconsin.

Barriers broken

Shusta's technique opens the door to large-scale preclinical studies of drug penetration into the brain. Potential applications include screening for therapeutic candidates that readily cross the BBB or

compounds that open the BBB to allow other therapeutic molecules to get across.

The *in vitro* system also could be used to study how the BBB opens up in neurological diseases such as MS, stroke and epilepsy. In these disorders, loss of BBB integrity is thought to contribute to brain damage, but the specific molecular players in pathological BBB opening have been hard to pin down.

Indeed, Shusta is now working on making brain endothelial cells from induced pluripotent stem cells from patients with MS and epilepsy.

According to Richard Daneman, research fellow in the Department of Anatomy at the **University of California, San Francisco**, prior *in vitro* BBB models required tradeoffs between physiological similarity to the human BBB, scalability and potency. Shusta's method scores high marks for all three, he said.

"The stem cell model here has several aspects that are better than all other models," said Daneman. "First, it's human—if you're trying to study drug delivery in humans, you want to use human tissue."

He noted that the method is also highly scalable and yields far more cells than was previously achieved with primary cell culture systems.

Daneman thinks the high potency of Shusta's cells arises from putting the stem cell precursors in an environment that resembles the embryonic brain.

In embryos, "these cells go through a lot of developmental stages to become brain endothelial cells," and Shusta's culture conditions mimic that environment, said Daneman.

Smaller, better, faster

Despite advantages in BBB potency and scalability, Shusta's stem cells may be harder to work with in an industrial setting than conventional BBB models that use transformed cells or primary cell culture because of the complexity of the culturing procedure, said Mohammad Kiani, professor and chair of mechanical engineering at **Temple University**.

Kiani also thinks the microfluidic platform for modeling the BBB and other functions of the microvasculature that he is developing will better replicate the role of blood flow at the BBB.

Kiani is collaborating with **CFD Research Corp.** (CFDRC) to develop a microfluidic platform for modeling the BBB and other functions of the microvasculature. Last month, CFDRC received a \$1.3 million grant from the **NIH** to further develop this technology, on which CFDRC holds patents.

Kiani said that growing endothelial and glial cells on separate parts of a microchip allows his team to study the effect of fluid flow on BBB functioning. He cited evidence that endothelial cells can regulate BBB activity in response to shear stress caused by blood flow.

Most *in vitro* BBB assays, including Shusta's, "are static systems where they look at cells or dyes going through a layer of cells. But that's not what happens in humans, where you have blood flow," said Kiani. "This makes a huge difference on the extravasation of particles and cells through the endothelium."

Thus, Kiani thinks *in vitro* systems that incorporate flow are "more physiological and in some cases fundamentally different than static conditions."

Shusta's assay of BBB activity involves traditional microtiter cell culture methods that do not easily scale up for high throughput

screening. However, combining Shusta's cells with Kiani's microfluidic chambers could yield a more physiological and scalable model of the BBB.

Kiani, Shusta and Daneman agreed that testing the behavior of stem cell-derived endothelial cells in a microfluidic system could answer basic questions about BBB mechanisms.

"The idea behind flow is that sheer stress can regulate influx, but it's an open question as to whether flow opens or closes the BBB," said Shusta.

With microfluidic systems, "the amount of drug you need to screen is low and the total number of cells you might use goes down," said Shusta.

He cautioned that one concern about using microfluidic systems is that the small number of cells in such a chamber may show higher variability than larger cell cultures.

"You have to be careful about interpreting drug permeability data from a small number of cells because cells are not all uniform" in their BBB activity, Shusta noted.

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Immunotherapy alliance for T1D

By Kai-Jye Lou, Staff Writer

Novo Nordisk A/S has partnered with **JDRF** to help populate the pharma's newly opened Type 1 Diabetes R&D Center in Seattle with early stage projects. The center will run side-by-side comparisons of technologies being developed through the foundation's sponsored projects as well as other opportunities the partners identify. A joint committee will select the most promising for further development by the pharma.

Novo Nordisk formed the Type 1 Diabetes R&D Center in January and opened its doors in June. The company plans to employ 20 immunotherapy researchers at the center, which is being led by Matthias von Herrath. He previously was director of the Center for Type

1 Diabetes Research at the **La Jolla Institute for Allergy & Immunology**.

Under a June deal, Novo Nordisk and JDRF will work together for at least two years and will focus on immunotherapies for type 1 diabetes (T1D). Initial research could include JDRF-funded projects from academic groups, biotechs and undisclosed programs from the pharma's internal research pipeline, as well as other opportunities identified by the partners.

For top candidates coming from academia or Novo Nordisk, the

partners will run additional preclinical and IND-enabling studies, after which they will decide whether to take the candidate into clinical trials. For these projects, clinical development will be driven primarily by the pharma. For candidates from biotech companies, the pharma will provide the company with resources to accelerate progress, such as funding support and access to the R&D center's facilities.

JDRF CSO Richard Insel said projects at the R&D center also could include external research that has not previously received funding from JDRF. He added that the partners also plan to look at technologies and therapies being developed for other diseases that could be repurposed for type 1 diabetes, especially immunotherapies for other autoimmune and allergic conditions.

Rights to existing IP for projects picked up under the collaboration will be retained by the project investigator. Licensing issues and rights to new IP generated from projects under the collaboration will be negotiated on a project-by-project basis.

Von Herrath said the deal establishes a system for the partners to build a pipeline of early stage technologies for type 1 diabetes and move them across the translational gap. "JDRF brings to the table its network of research partners from academia and industry and the supporting databases of information it has on the history and natural progression of type 1 diabetes," he told *SciBX*.

JDRF's databases include information from both clinical and preclinical studies.

"JDRF doesn't have its own laboratories, so we haven't had a good way to make direct comparisons between research projects evaluating different therapeutic approaches for type 1 diabetes," added Insel. "Novo Nordisk's Type 1 Diabetes R&D Center will let us assess and make side-by-side comparisons on the scientific validity, technical feasibility and commercial viability of various immunotherapeutic strategies being developed for type 1 diabetes."

Milestones for the Novo Nordisk-JDRF deal and details of specific targets, compounds and projects are undisclosed. The pharma has not disclosed any immunotherapy programs for type 1 diabetes in its pipeline.

The partners have not yet selected any projects for the R&D center and are not disclosing when they plan to start the first comparison studies. They are not providing metrics on the number of candidates they expect to compare and select for further development once the operation is ramped up.

Although specific financial details of the R&D collaboration are not being disclosed, von Herrath said the flow of money "will be from Novo Nordisk to JDRF and not the other way around."

Funding for existing JDRF-sponsored research projects not selected for development at the R&D center will not be affected.

A space with opportunities

Von Herrath said one of the key obstacles in developing immunotherapies for type 1 diabetes is the need to measure up to the safety and efficacy of available chronic therapies, such as insulin. Indeed, broadly immunosuppressive drugs such as cyclosporine A cannot be chronically used in the indication because they leave patients highly susceptible to infection and their long-term use increases the risk of cancer.

Thus, von Herrath said one class of immunotherapies the partners will consider is antigen-specific vaccines.

"Antigenic vaccines work by augmenting regulatory T cell activity and have site-specific activity," he told *SciBX*. "These properties make them very attractive for use in type 1 diabetes as they locally suppress both general inflammation and the immune response against the targeted autoantigen without causing systemic immunosuppression."

T_{reg} cells suppress the activity of autoreactive T cells, which attack and destroy insulin-producing pancreatic β cells in type 1 diabetes.

The partners also are interested in picking up projects related to biomarkers that can be used to predict and evaluate treatment response and monitor disease progression.

Von Herrath noted that such projects could, for example, include developing a bioinformatics platform to parse the information contained within JDRF's databases and other databases. These projects could lead to better understanding of disease progression and aspects of the disease that could be targeted with immunotherapies and also could help better tailor therapies to the optimal patient group, he said.

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COMPANIES AND INSTITUTIONS MENTIONED

JDRF, New York, N.Y.

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"JDRF brings to the table its network of research partners from academia and industry and the supporting databases of information it has on the history and natural progression of type 1 diabetes."

—Matthias von Herrath,
Novo Nordisk A/S

More druggable than MYC

By Lauren Martz, Staff Writer

A Fred Hutchinson Cancer Research Center group has used a cell-based screen to identify 12 potential therapeutic targets in MYC-driven cancers that could be more druggable than MYC.¹ The researchers hope to find an industry or academic partner to help develop inhibitors of some of the targets.

Aberrant overexpression of *c-Myc* (*MYC*) occurs in a variety of cancers. For example, 50% of ovarian cancers and 30% of hepatocellular carcinomas are driven by *MYC* overexpression.^{2,3}

But the structure of MYC lacks any druggable domains, and as a result it has been impossible to design compounds that cleanly hit the protein. Previous studies have suggested other nonmutated genes and signaling pathways that are active within cancer cells and are required for survival of MYC-driven malignancies.⁴ However, only a handful of druggable targets, such as cyclin dependent kinases (CDKs), have been identified by these efforts so far.⁵

In addition to these screening efforts, two papers have also recently implicated bromodomain containing 4 (BRD4) as a potential therapeutic target in MYC-driven cancers. A team from Cold Spring Harbor Laboratory and Dana-Farber Cancer Institute and a team from GlaxoSmithKline plc, Cellzome AG and the UK independently showed evidence for efficacy of BRD4 inhibitors in mouse models of leukemias characterized by MYC activation.^{6,7}

The Fred Hutchinson team, led by Carla Grandori, has now developed an additional screening approach to identify new pathways and genes that could serve as therapeutic targets for cancers that overexpress MYC.

The team screened a small interfering RNA library that targeted about 3,300 genes encoding known cancer-associated proteins, including tyrosine kinases, ubiquitin ligases, DNA repair proteins and other known druggable targets.

The screen identified 148 genes that reduced cell survival following siRNA knockdown in MYC-overexpressing human fibroblasts. Based on predicted druggability, potential involvement in cancer pathways and potential toxicity, the researchers pared the list down to 48.

Next, the researchers looked at the 48 genes in a cell culture model of MYC-driven neuroblastoma. In those cells, siRNA against 12 of the targets selectively induced cell death.

Among the 12 candidates, the team selected casein kinase 1ε (CSNK1E; CKI-ε) for the remainder of the study because its knockdown potently and selectively inhibited MYC-amplified cancer cell survival and because research tool inhibitors of the protein are available.

In mice with human neuroblastoma xenografts, a CSNK1E inhibitor decreased tumor growth compared with vehicle control.

The findings were published in the *Proceedings of the National Academy of Sciences*. The paper also included researchers from the University of Washington.

“This screening approach shows that we can target nonmutated genes that are essential for the expression and function of MYC,” Grandori told *SciBX*. Grandori is a research member at Fred Hutchinson.

“As MYC is a nondruggable target as far as small molecules go and other approaches such as oligonucleotides or RNAi have either failed or have the drawbacks of biologics, the approach described by the authors, as well as others, is very elegant and offers a way around the druggability issue,” said Dominique Cheneval, founder, president and COO of Novation Pharmaceuticals Inc.

The others include a group from Baylor College of Medicine, who earlier this year used a similar approach with an RNAi library to identify pathways that support MYC-driven cancers. The team identified and focused on genes involved in SUMOylation including *SUMO1 activating enzyme subunit 1* (*SAE1*) and *SAE2* (*UBA2*) as potential druggable targets.⁸

Novation has a cell-based assay technology to identify small molecules that influence mRNA stability and translation. The company

has identified MYC inhibitors that alter MYC mRNA expression and translation using the technology, but it has not released functional data.

Teaming up

The Fred Hutchinson group now has 11 targets “including kinases and proteins with known druggable domains that could be very promising for MYC-amplified cancers,” Grandori told *SciBX*. “We are doing additional bioinformatics and validation experiments for some of the targets, including CSNK1E, but we can’t go after them all.”

Thus, the team’s next hope is to partner with a company or academic institution to develop and test inhibitors of CSNK1E and other validated targets for drug discovery.

Grandori also believes that the importance of her study extends beyond the identification of potential therapeutic targets for MYC-driven cancers.

She noted that her approach is unique from other cancer-directed siRNA screening efforts because it uses a different cell system. “Many discovery screens in use today utilize genetically and phenotypically diverse human cancer cell lines that have been cultured for years and then resort to bioinformatics to infer synthetic lethal interactions. It is clear that there is too much noise,” said Grandori.

Her team utilized human foreskin fibroblasts that were engineered to allow researchers to study the effects of a single oncogene at a time, and unlike other noncancerous cells, this cell line is able to express oncogenes without losing cell viability, she added.

Grandori told *SciBX* that Fred Hutchinson has filed patent applications for the use of inhibitors toward CSNK1E and provisional patent applications for the other identified targets. The IP is available for licensing. The screening technology has not been patented.

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—Dominique Cheneval,
Novation Pharmaceuticals Inc.

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Crossing the mucus barrier

By Tracey Baas, Senior Editor

A team from **The Johns Hopkins University** has shown that vaginal delivery of antiviral-loaded, mucus-penetrating particles can help prevent herpes simplex virus infection in mice.¹ The team thinks the particles have potential utility in infection, cancer, inflammation and other diseases that affect mucosal tissues in the eyes, sinuses, female reproductive tract, respiratory tract and GI tract.

Kala Pharmaceuticals Inc. is the exclusive worldwide licensee of the IP from Johns Hopkins University and is using the technology to develop improved treatments for mucosal tissue diseases, including cystic fibrosis (CF), severe ocular inflammation and ulcerative colitis.

In 2000, the Johns Hopkins group set out to develop nanoparticles that could cross mucus layers and release compounds. To do so, the group based the design of the particles' outside surface on the surfaces of viruses that can penetrate the mucus barrier. The team coated conventional biodegradable nanoparticles with high concentrations of low-molecular weight polyethylene glycol (PEG) and showed that the resulting particles could diffuse through human cervicovaginal mucus (CVM) and also through sputum from the lungs of patients with CF.²⁻⁴

The unanswered question was whether the mucus-penetrating particles provided broad distribution and long-term retention *in vivo* in the vagina—two factors that have stymied prior nanoparticle technologies.

The first step was developing an improved mouse model for vaginal delivery studies because both mice and nonhuman primate CVM have different characteristics than human CVM. The team used an estradiol-based treatment to generate mice that produced CVM that more closely resembled the CVM of humans.

"The treatment induces a state that occurs naturally in the mouse estrous cycle in which the vaginal tissue and mucus are similar to those of humans," said Laura Ensign-Hodges, chemical and biomolecular engineering graduate student at Johns Hopkins and lead author of the paper in *Science Translational Medicine* that described the findings. "We think the mouse model provides a better system to evaluate vaginal delivery than previous mouse models."

"The old mouse model predicted toxicity associated with previous microbicide products that were not evident in humans until Phase III. However, the hormonal treatment used in the old model makes the mucus and the epithelium of the mouse vagina dissimilar from that of the human vagina, so other vitally important characteristics of a vaginal formulation such as distribution, retention and uptake should be studied with the new mouse model," added team member Richard Cone, a professor of biophysics and biology at Johns Hopkins.

In the model, nanoparticles delivered to the vagina formed a continuous layer that coated the entire epithelium, even within deep vaginal folds. Moreover, 60% of the particles were retained for up to 6

hours. In contrast, conventional nanoparticles aggregated in the luminal mucus and only 10% were retained for 6 hours.

Particles loaded with a fluorescent compound delivered payload to at least 87% of the vaginal surface and were retained even after 24 hours, whereas the fluorescent compound formulated in a standard vaginal hydroxyethylcellulose gel delivered payload to 42% of the vaginal surface.

The researchers also took a close look at whether their nanoparticles caused the vaginal epithelium to secrete cytokines and immune mediators that may enhance susceptibility to sexually transmitted infections—a known potential risk of microbicides such as nonoxynol-9.

In the mice, nonoxynol-9 or conventional nanoparticles both led to an influx of neutrophils, whereas the mucus-penetrating particles did not. In addition, levels of proinflammatory cytokines that are associated with epithelial injury were increased after repeat dosings of nonoxynol-9 but not after repeated dosings of mucus-penetrating particles.

Finally, the researchers wanted to show that the loaded mucus-penetrating particles could prevent herpes simplex virus (HSV) infection in mice. Animals pretreated with nanoparticles loaded with the generic HSV drug acyclovir showed 53% protection, whereas mice pretreated with soluble acyclovir showed 12% protection.

Thomas Moench, president and COO of **ReProtect Inc.**, said acyclovir was a good choice for the study because it has notoriously poor efficacy in the prophylactic setting when given in solution. The next step, he said, should be to test the nanoparticles with a more potent drug.

Team leader Justin Hanes agreed. "Our focus with this work was to show that mucus-penetrating particles enhanced efficacy and did not cause toxicity or inflammation that could enhance virus infection. In this study, we weren't looking for perfect or complete protection," he said. "We specifically selected acyclovir because it has poor efficacy even though it is the best available. This way we could test whether the particles improved antiviral efficacy."

Hanes is professor of ophthalmology in the Wilmer Eye Institute at Johns Hopkins and director of the Center for Nanomedicine at **The Johns Hopkins University School of Medicine**. He is a cofounder and director of Kala.

"Our focus with this work was to show that mucus-penetrating particles enhanced efficacy and did not cause toxicity or inflammation that could enhance virus infection."

—Justin Hanes,
The Johns Hopkins University

Eyeing indications

Kevin Pojasek, VP of corporate development at Kala, said the company is using its mucosa-penetrating platform to engineer medicines for treating diseases affecting mucosal tissues. Kala's internal programs are in preclinical development, and the company also will seek deals to apply the technology platform to partners' compounds.

Meanwhile, Hanes first wants to test nanoparticles loaded with Viread tenofovir in a mouse model of HIV. The Hopkins team also is developing mucus-penetrating nanoparticles to deliver chemotherapeutics for lung and cervical cancers, anti-inflammatories for lung inflammation and chronic rhinosinusitis, microbicides for rectal use and gene therapy for CF.

Gilead Sciences Inc. markets the reverse transcriptase inhibitor Viread to treat HIV infection. The company is running a Phase IIb trial of tenofovir 1% vaginal gel to prevent HIV infection.

Hanes previously collaborated with **Copernicus Therapeutics Inc.** to develop DNA-carrying nanoparticles to deliver gene therapy for CF and ophthalmic disease.

“The nanoparticle formulation consists of single molecules of DNA compacted with polyethylene glycol–substituted lysine peptides,” said Mark Cooper, SVP of science and medical affairs at Copernicus. “Additional nanoparticle refinements, such as optimization of PEG size and density, permit DNA nanoparticle diffusion through mucus, as pioneered by Hanes. The small size of our particles permits transit through the nuclear membrane pore, allowing delivery of therapeutic genes.”

Copernicus’ nanoparticle-delivered gene therapy to treat CF is in Phase I/II testing.

Johns Hopkins holds a European patent covering the work reported in *Science Translational Medicine* and has filed for a patent in the U.S. The IP is licensed to Kala.

Baas, T. *SciBX* 5(27); doi:10.1038/scibx.2012.697
Published online July 12, 2012

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Contact: Justin Hanes, The Johns Hopkins University School of Medicine, Baltimore, Md.
e-mail: hanes@jhu.edu
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3. Lai, S.K. *et al. Proc. Natl. Acad. Sci. USA* **104**, 1482–1487 (2007)
4. Tang, B.C. *et al. Proc. Natl. Acad. Sci. USA* **106**, 19268–19273 (2009)

COMPANIES AND INSTITUTIONS MENTIONED

Copernicus Therapeutics Inc., Cleveland, Ohio
Gilead Sciences Inc. (NASDAQ:GILD), Foster City, Calif.
The Johns Hopkins University, Baltimore, Md.
The Johns Hopkins University School of Medicine, Baltimore, Md.
Kala Pharmaceuticals Inc., Waltham, Mass.
ReProtect Inc., Baltimore, Md.

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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				
Acute myelogenous leukemia (AML); myelodysplastic syndrome (MDS)	IL-1 receptor accessory protein (IL-1RAP)	<i>In vitro</i> and human studies suggest IL-1RAP could be a prognostic marker of AML and MDS. In hematopoietic stem cells and granulocyte-monocyte progenitor cells from patients with AML and MDS, IL-1RAP levels were greater than those in healthy controls. In a cohort of patients with AML, high IL-1RAP expression was associated with poor overall survival ($p=2.2 \times 10^{-7}$). Ongoing work includes investigating IL-1RAP as a prognostic marker in chronic myelogenous leukemia (CML) and as marker of leukemic stem cells. Anti-IL-1RAP, a mAb against IL-1RAP from Cantargia AB, is in preclinical development to treat melanoma. SciBX 5(27); doi:10.1038/scibx.2012.698 Published online July 12, 2012	Patented by the Albert Einstein College of Medicine of Yeshiva University; available for licensing or partnering	Barreyro, L. <i>et al. Blood</i> ; published online June 21, 2012; doi:10.1182/blood-2012-01-404699 Contact: Ulrich Steidl, Albert Einstein College of Medicine of Yeshiva University, Bronx, N.Y. e-mail: ulrich.steidl@einstein.yu.edu
Breast cancer	Membrane-associated guanylate kinase WW and PDZ domain containing 3 (MAGI3); protein kinase B γ (PKB γ ; AKT3)	Patient sample and cell culture studies suggest AKT (PKB; PKBA; AKT1) inhibitors could help treat breast cancers expressing the MAGI3-AKT3 fusion protein. Genome sequencing and computational analysis identified a MAGI3-AKT3 fusion protein in 5 of 72 triple-negative tumor samples. In cell culture, expression of MAGI3-AKT3 led to excess phosphorylation of AKT substrates, which was inhibited by a pan-AKT inhibitor. Next steps include additional cellular and animal model experiments. At least 11 companies have AKT inhibitors in development from Phase I to Phase III trials for various types of cancer. SciBX 5(27); doi:10.1038/scibx.2012.699 Published online July 12, 2012	Patent application filed; unlicensed	Banerji, S. <i>et al. Nature</i> ; published online June 20, 2012; doi:10.1038/nature11154 Contact: Matthew Meyerson, Dana-Farber Cancer Institute, Boston, Mass. e-mail: matthew_meyerson@dfci.harvard.edu
Cancer; breast cancer	Deoxyuridine triphosphate (dUTP)	<i>In vitro</i> and mouse studies suggest a class of dUTP inhibitors could help treat cancer. Chemical synthesis and <i>in vitro</i> testing of 1,2,3-triazole-containing uracil analogs identified a lead compound as a low nanomolar dUTP inhibitor. In a cancer cell line, the compound and 5-fluorouracil (5-FU) increased cell death compared with either agent alone. In mice with xenograft breast tumors, the compound plus 5-FU was safe and decreased tumor growth compared with either agent alone. Future studies could include testing the lead compound in animal models of other cancers. The generic 5-FU is marketed to treat colorectal, pancreatic and other cancers. SciBX 5(27); doi:10.1038/scibx.2012.700 Published online July 12, 2012	Patented by Taiho Pharmaceutical Co. Ltd.; unavailable for licensing	Miyakoshi, H. <i>et al. J. Med. Chem.</i> ; published online June 20, 2012; doi:10.1021/jm3004174 Contact: Masayoshi Fukuoka, Taiho Pharmaceutical Co. Ltd., Tsukuba, Japan e-mail: m-fukuoka@taiho.co.jp

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Colorectal cancer	Epidermal growth factor receptor (EGFR); MEK; <i>K-Ras</i>	Cell culture and patient sample studies suggest deep sequencing of circulating tumor DNA can detect early resistance to anti-EGFR drugs and help guide rational combination therapies. In a cellular model of Erbitux cetuximab resistance, deep sequencing identified small subpopulations of Erbitux-resistant <i>K-Ras</i> mutants before treatment and secondary <i>K-Ras</i> mutants post-treatment that responded to MEK inhibitors plus Erbitux. In biopsies from 10 patients with colorectal cancer that became refractory to the anti-EGFR mAbs Erbitux or Vectibix panitumumab, deep sequencing of circulating tumor DNA identified <i>K-Ras</i> mutations in patient plasma as early as 10 months before radiology showed tumor progression. Next steps include determining other mechanisms of acquired resistance and using patient-derived tumor xenografts to validate the cell-based findings. Eli Lilly and Co., Bristol-Myers Squibb Co. and Merck KGaA market Erbitux to treat colorectal cancer and head and neck cancer. Amgen Inc. and Takeda Pharmaceutical Co. Ltd. market Vectibix to treat metastatic colorectal cancer. SciBX 5(27); doi:10.1038/scibx.2012.701 Published online July 12, 2012	Patent application filed; available for licensing	Misale, S. <i>et al. Nature</i> ; published online June 13, 2012; doi:10.1038/nature11156 Contact: Alberto Bardelli, Institute for Cancer Research and Treatment, Candiolo, Italy e-mail: alberto.bardelli@irc.it
Cardiovascular disease				
Cardiovascular disease	Potassium channel Kv11.1 (KCNH2)	Cell culture studies suggest increasing KCNH2 activity could help prevent long QT syndrome (LQTS). In induced pluripotent stem (iPS) cell-derived cardiomyocytes from patients with LQTS, a chemical activator of human KCNH2 decreased excessively long action potential duration compared with no treatment. Next steps include improving the drug-like properties of the KCNH2 activator and profiling its activity and safety. SciBX 5(27); doi:10.1038/scibx.2012.702 Published online July 12, 2012	Patent pending; available for licensing	Zhang, H. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 28, 2012; doi:10.1073/pnas.1205266109 Contact: Min Li, The Johns Hopkins University, Baltimore, Md. e-mail: minli@jhmi.edu
Myocardial infarction (MI)	Myosin heavy chain 6 cardiac muscle- α (MYH6)	Mouse studies suggest blocking autoimmune activity against cardiac myosin could help reduce damage following MI in patients with type 1 diabetes. In nonobese diabetic (NOD) mice, experimentally induced MI led to production of autoantibodies against cardiac myosin that caused cardiac tissue destruction and inflammation. Tolerance to Myh6 prevented the increase in symptom severity following MI. In post-MI patients, autoantibodies against cardiac antigens were identified in 15 of 18 patients with type 1 diabetes but in only 3 of 20 patients with type 2 diabetes. Next steps include developing antigen-specific immunotherapeutics for delivery after heart attack. SciBX 5(27); doi:10.1038/scibx.2012.703 Published online July 12, 2012	Patent application filed; available for licensing	Gottumukkala, R.V.S.R.K. <i>et al. Sci. Transl. Med.</i> ; published online June 13, 2012; doi:10.1126/scitranslmed.3003551 Contact: Myra A. Lipes, Harvard Medical School, Boston, Mass. e-mail: myra.lipes@joslin.harvard.edu
Endocrine/metabolic disease				
Diabetes	Histone deacetylase 3 (HDAC3)	A study in cell culture suggests inhibiting HDAC3 could help treat type 1 diabetes. In a rat β cell line, knockdown of Hdac3 decreased activation of caspase-3 (Casp3; Cpp32), a mediator of type 1 diabetes-associated apoptosis, compared with no knockdown. Next steps include developing selective HDAC3 inhibitors to test in cellular and mouse models of diabetes. Repligen Corp.'s RG2833, a selective HDAC3 inhibitor, is in Phase I testing in ataxia. SciBX 5(27); doi:10.1038/scibx.2012.704 Published online July 12, 2012	Unpatented; licensing status not applicable	Chou, D.H.-C. <i>et al. Chem. Biol.</i> ; published online June 22, 2012; doi:10.1016/j.chembiol.2012.05.010 Contact: Bridget K. Wagner, Broad Institute of MIT and Harvard, Cambridge, Mass. e-mail: bwagner@broadinstitute.org

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Obesity	Ghrelin receptor (GHSR)	<i>In vitro</i> and rodent studies identified a peptide antagonist of GHSR that could help treat obesity. An <i>in vitro</i> screen of a cDNA display library identified the peptide. In fasting mice, i.v. delivery of the peptide decreased food intake compared with vehicle. Next steps include improving the peptide's potency. AEZS-123, a GHSR antagonist from Aeterna Zentaris Inc., is in preclinical development for obesity. EX-1350, a GHSR antagonist from Elixir Pharmaceuticals Inc., is in preclinical development for diabetes and obesity.	Patent pending; licensing status undisclosed	Ueno, S. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 20, 2012; doi:10.1073/pnas.1203561109 Contact: Takafumi Sakai, Saitama Industrial Technology Center, Saitama, Japan e-mail: tsakai@mail.saitama-u.ac.jp
Infectious disease				
Gram-negative bacterial infection	Pesticin receptor protein (FyuA)	<i>In vitro</i> studies identified a modified variant of pesticin that could help treat Gram-negative bacterial infections. Pesticin is a toxic protein that kills Gram-negative bacteria expressing FyuA, although many strains encode a gene that confers pesticin resistance. Crystallography and bacterial growth assays were used to engineer a hybrid pesticin-lysozyme peptide that killed FyuA-expressing bacteria carrying plasmid-encoded pesticin resistance. Next steps include optimizing the toxin's stability for <i>in vivo</i> experiments.	Patent and licensing status undisclosed	Lukacik, P. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 7, 2012; doi:10.1073/pnas.1203472109 Contact: Susan K. Buchanan, National Institutes of Health, Bethesda, Md. e-mail: skbuchan@helix.nih.gov
Neurology				
Huntington's disease (HD)	Huntingtin (HTT)	<i>In vitro</i> and mouse studies suggest stem cell therapy could help treat HD. In induced pluripotent stem (iPS) cells derived from HD patient fibroblasts, genetic replacement of the HD-associated CAG repeats in HTT generated pluripotent cells that differentiated into neuronal stem cells (NSCs) with no disease phenotype. In a mouse model of HD, transplantation of the corrected NSCs into the striatum repopulated the mouse brains with healthy human neurons. Next steps include correcting iPS cells from additional HD patients and working with the HD patient-derived iPS cells to screen for drug leads.	Unpatented; available for licensing	An, M.C. <i>et al. Cell Stem Cell</i> ; published online June 28, 2012; doi:10.1016/j.stem.2012.04.026 Contact: Lisa M. Ellerby, Buck Institute, Novato, Calif. e-mail: ellerby@buckinstitute.org
Neurology	Suppressor of cytokine signaling 3 (SOCS3)	<i>In vitro</i> studies suggest the cardiovascular drug Lopid gemfibrozil could be repurposed to treat neuroinflammatory and neurodegenerative diseases. In mouse microglia or astrocytes, Lopid dose-dependently increased levels of Socs1, Socs2 and Socs3 mRNA, which protect against neuronal inflammation, compared with vehicle. Ongoing work includes testing gemfibrozil in an animal model of multiple sclerosis (MS), and next steps include testing the compound in other animal models of neurodegenerative diseases. Pfizer Inc. markets Lopid to treat hypercholesterolemia and coronary artery disease (CAD).	Unpatented; available for licensing	Ghosh, A. & Pahan, K. <i>J. Biol. Chem.</i> ; published online June 8, 2012; doi:10.1074/jbc.M112.346932 Contact: Kalipada Pahan, Rush University Medical Center, Chicago, Ill. e-mail: kalipada_pahan@rush.edu

This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Ophthalmic disease				
Retinal detachment	Mineralocorticoid receptor	Rat and human studies suggest antagonizing the mineralocorticoid receptor could help treat central serous chorioretinopathy (CSCR). CSCR involves dilation and leakage of choroid vessels under the retina, which causes retinal detachment. In rats, intravitreal injection of the mineralocorticoid receptor activator aldosterone induced choroid vessel dilation and leakage. In two patients with chronic CSCR, the mineralocorticoid receptor antagonist eplerenone decreased subretinal fluid retention compared with that seen prior to treatment and resolved retinal detachment for up to five months following treatment withdrawal. Next steps include clinical testing and development of a new formulation of the compound. Pfizer Inc. markets Inspra eplerenone to treat hypertension and myocardial infarction (MI).	Patent application filed; available for licensing	Zhao, M. <i>et al. J. Clin. Invest.</i> ; published online June 11, 2012; doi:10.1172/JCI61427 Contact: Nicolette Farman, Institut National de la Santé et de la Recherche Médicale (INSERM) U872 Cordelier Research Center, Paris, France e-mail: nicolette.farman@crc.jussieu.fr
SciBX 5(27); doi:10.1038/scibx.2012.709 Published online July 12, 2012				
Various				
Blood substitute; hypoxia	Not applicable	Studies in human blood and rabbits suggest oxygen-loaded microparticles could help treat asphyxia and other hypoxemic conditions. <i>In vitro</i> , phospholipid- and copolymer-based microparticles transferred free oxygen to the hemoglobin of deoxygenated human red blood cells within four seconds without altering blood viscosity. In a rabbit model of hypoxemia and asphyxia, the microparticles normalized blood levels of oxygenated hemoglobin and decreased metabolic acidosis, signs of liver injury and incidence of cardiac arrest compared with control particles not loaded with oxygen. Ongoing work includes testing the microparticles in animal models of cardiac arrest.	Patented; licensing status undisclosed	Kheir, J.N. <i>et al. Sci. Transl. Med.</i> ; published online June 27, 2012; doi:10.1126/scitranslmed.3003679 Contact: John N. Kheir, Boston Children's Hospital, Boston, Mass. e-mail: john.kheir@childrens.harvard.edu
SciBX 5(27); doi:10.1038/scibx.2012.710 Published online July 12, 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
Assays & screens			
<i>Drosophila</i> cancer models to identify inhibitors that block an optimized combination of kinase targets	<p>Screening in <i>Drosophila</i> cancer models could aid the identification of inhibitors against an optimized combination of kinase targets. In a <i>Drosophila</i> model of multiple endocrine neoplasia type 2 (MEN2), a disease caused by activating mutations in ret proto-oncogene (RET), screening a library of small molecules that inhibited RET and other kinases identified a lead molecule that caused some flies to survive to adulthood. In the <i>Drosophila</i> model, the initial hit was optimized to block a combination of kinases to increase efficacy and decrease toxicity. In MEN2 mice, the lead molecule decreased tumor growth compared with Caprelsa vandetanib. Next steps include further optimizing the inhibitor and screening kinase inhibitor libraries in <i>Drosophila</i> models of additional cancers.</p> <p>Caprelsa, an inhibitor of RET and other kinases from AstraZeneca plc, is approved to treat advanced medullary thyroid cancer, a disease that can result from MEN2.</p> <p>SciBX 5(27); doi:10.1038/scibx.2012.711 Published online July 12, 2012</p>	Patent application filed; licensing discussions ongoing	<p>Dar, A.C. <i>et al. Nature</i>; published online June 6, 2012; doi:10.1038/nature11127 Contact: Kevan M. Shokat, University of California, San Francisco, Calif. e-mail: shokat@cmp.ucsf.edu</p>
Modification of DNA abasic (AP) sites to help detect AP lesions with nanopore sequencing	<p>The 2-aminomethyl-18-crown-6 (18c6) modification of DNA AP sites could help detect AP lesions during nanopore sequencing. AP sites are among the most frequent lesions in the genome and have high mutagenic potential. DNA modified with 18c6 moved more slowly through the bacterial protein ion channel α-hemolysin nanopore than unmodified DNA and produced a current amplitude signature that enabled detection of the AP site. Next steps include using the method to screen for AP sites in clinical samples and developing other methods to modify DNA AP sites.</p> <p>SciBX 5(27); doi:10.1038/scibx.2012.712 Published online July 12, 2012</p>	Patent application filed; available for licensing	<p>An, N. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online June 18, 2012; doi:10.1073/pnas.1201669109 Contact: Cynthia J. Burrows, The University of Utah, Salt Lake City, Utah e-mail: burrows@chem.utah.edu Contact: Henry S. White, same affiliation as above e-mail: white@chem.utah.edu</p>
Computational models			
Computational strategy for predicting mechanism of action for drugs and probes	<p>A computational strategy could be useful for predicting the mechanism of action and molecular targets of existing drugs and probes. The strategy, called the similarity ensemble approach (SEA), predicted a candidate molecule's affinity for a given target based on the molecule's structural similarity to known ligands of the target. The strategy predicted the molecular target of 59 of 78 drugs in a database. In two additional databases, the strategy plus literature searches predicted the molecular target of 308 of 352 and 340 of 556 drugs, respectively. Next steps include using the computational strategy to help predict the molecular targets for lead candidates emerging from phenotypic screens. SeaChange Pharmaceuticals Inc. is developing software that uses the SEA.</p> <p>SciBX 5(27); doi:10.1038/scibx.2012.713 Published online July 12, 2012</p>	Predicted interactions and SEA unpatented; unlicensed; software developed by SeaChange Pharmaceuticals protected by trademark and copyright; software available for licensing	<p>Gregori-Puigjané, E. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online June 18, 2012; doi:10.1073/pnas.1204524109 Contact: Brian K. Shoichet, University of California, San Francisco, Calif. e-mail: bshoichet@gmail.com Contact: Bryan L. Roth, The University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, N.C. e-mail: bryan_roth@med.unc.edu</p>

This week in techniques

Approach	Summary	Licensing status	Publication and contact information
Drug platforms			
Cell-free biodegradable elastomeric grafts to promote artery formation	Grafts made of a biodegradable elastomeric material could be useful for promoting artery formation. The grafts consisted of a heparin-coated porous tube made of poly(glycerol sebacate) sheathed in a polycaprolactone mesh. In rats, degradation of the graft led to the formation of new arteries that integrated with surrounding host tissue over three months. Those arteries were structurally and functionally similar to native aortic arteries. Next steps include optimizing and standardizing the graft fabrication processes and evaluating the graft in large animal models. SciBX 5(27); doi:10.1038/scibx.2012.714 Published online July 12, 2012	Patent application filed; available for licensing from the University of Pittsburgh Office of Technology Management	Wu, W. <i>et al. Nat. Med.</i> ; published online June 24, 2012; doi:10.1038/nm.2821 Contact: Yadong Wang, University of Pittsburgh, Pittsburgh, Pa. e-mail: yaw20@pitt.edu
Markers			
Genomic markers to guide the use of neoadjuvant aromatase inhibitors in patients with breast cancer	Whole-genome and exome sequencing of pretreatment tumor biopsies from patients with breast cancer that received neoadjuvant aromatase inhibitors identified potential markers for patient stratification. A predictive algorithm based on the sequencing and clinical data determined that individuals with <i>MAP kinase kinase 1 (MAP3K1; MEK1)</i> mutations, low Ki-67 (antigen KI-67; MKI67) index and luminal A subtype likely would respond to neoadjuvant aromatase inhibitor treatment, whereas those with <i>p53</i> mutations, high Ki-67 index and luminal B subtype likely would not respond. Next steps include using larger numbers of patient samples to optimize the predictive algorithm in preparation for using the method to stratify patients in a clinical trial. Marketed aromatase inhibitors for breast cancer include AstraZeneca plc's Arimidex anastrozole, Novartis AG's Femara letrozole and Pfizer Inc.'s Aromasin exemestane. SciBX 5(27); doi:10.1038/scibx.2012.715 Published online July 12, 2012	Unpatented; licensing status not applicable	Ellis, M.J. <i>et al. Nature</i> ; published online June 10, 2012; doi:10.1038/nature11143 Contact: Elaine R. Mardis, Washington University in St. Louis, St. Louis, Mo. e-mail: emardis@wustl.edu
Membrane proteins anchored by glycosylphosphatidylinositol as markers of breast carcinomas	Membrane proteins anchored by glycosylphosphatidylinositol could be useful markers of breast carcinomas. In human cell lines, <i>Clostridium septicum</i> α -toxin bound and captured glycosylphosphatidylinositol-anchored membrane proteins. Human breast cancer tissues showed greater α -toxin binding than normal breast tissue. In human sera samples, two of the identified α -toxin-binding proteins, fermitin family member 3 (FERMT3) and filamin A (FLNA), were detected in at least 90% of breast cancer cases, whereas they were detected in 10% and 0% of nonmalignant sera, respectively. Next steps could include validating the candidate breast cancer biomarkers by analyzing sera samples from patients and nondiseased controls. SciBX 5(27); doi:10.1038/scibx.2012.716 Published online July 12, 2012	Patent application filed covering use of α -toxin as a tool for the discovery and detection of cancer biomarkers; available for licensing from The University of Georgia Technology Commercialization Office	Zhao, P. <i>et al. J. Cell Biol.</i> ; published online May 31, 2012; doi:10.1074/jbc.M112.339465 Contact: Karen L. Abbott, Complex Carbohydrate Research Center, The University of Georgia, Athens, Ga. e-mail: kabbott@uga.edu
Pseudogenes as markers and therapeutic targets for human diseases	Transcriptome analysis and <i>in vitro</i> studies suggest pseudogenes could be useful as biomarkers or therapeutic targets for human diseases such as cancer. Pseudogenes are copies of genes that have lost the ability to encode proteins. Transcriptome analysis of 248 cancer and 45 benign samples covering 13 tissue types generated pseudogene expression maps that helped identify tissue- and cancer-specific pseudogenes. In two human breast cancer cell lines, small interfering RNA-mediated knockdown of a breast cancer-associated pseudogene decreased proliferation compared with knockdown of the wild-type version of the gene. Next steps could include studies in larger numbers of samples. SciBX 5(27); doi:10.1038/scibx.2012.717 Published online July 12, 2012	Patent and licensing status undisclosed	Kalyana-Sundaram, S. <i>et al. Cell</i> ; published online June 22, 2012; doi:10.1016/j.cell.2012.04.041 Contact: Arul M. Chinnaiyan, University of Michigan, Ann Arbor, Mich. e-mail: arul@umich.edu

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