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S-nitrosylation boon to blood transfusions

By Kai-Jye Lou, Senior Writer

Red blood cell transfusions are among the most commonly performed medical procedures, but the capacity of the transfused cells to transport oxygen is often reduced because of biochemical changes in the cells during storage. Now, **Case Western Reserve University** and **Duke University Medical Center** researchers have improved oxygen delivery capacity by re-nitrosylating hemoglobin prior to transfusion.¹

The researchers are planning a series of clinical trials to determine whether the procedure can decrease the incidence of transfusion-related adverse events compared with the current standard of care and potentially improve patient outcomes.

Red blood cell (RBC) transfusions are intended to improve tissue oxygenation based on the known correlation between the oxygen carrying capacity of blood and oxygen delivery to tissues. Transfusions improve the oxygen carrying capacity of the recipient's blood by increasing the number of RBCs.

However, stored RBCs are inferior to fresh RBCs at delivering oxygen because of physical and biochemical changes that occur in the cells during storage.² Multiple groups also have found that RBC transfusions can do the exact opposite of what is intended and actually decrease tissue oxygenation, possibly because of these storage-induced changes.^{3,4}

Other studies have associated RBC transfusions with negative outcomes in some indications such as myocardial infarction (MI) and in the cardiac surgery setting,⁵⁻⁸ although it is unclear whether diminished oxygen delivery is the culprit.

In 2007, a group led by Jonathan Stamler reported that banked RBCs have a deficiency in S-nitrosohemoglobin levels. In a canine model, his team found that correcting the deficiency with a process called S-nitrosylation increased both the vasodilatory activity of RBCs and blood flow to tissues compared with what was seen in stored, untreated blood.⁹ Blood flow is a key determinant of oxygen delivery to tissues.

"The reality is that oxygen content in blood appears to be separate

"Most practitioners consider fresh banked blood very efficacious, but the authors are arguing that since even the freshest blood from the blood bank has lost most of its S-nitrosohemoglobin, even these blood units could be made more effective by re-nitrosylation."

—John Roback,
Emory University School of
Medicine

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and distinct from its ability to deliver oxygen to organs and tissues,” said Stamler, a professor of medicine and director of the Institute for Transformative Molecular Medicine at Case Western. “We have come to understand and are making the case that the delivery of oxygen to tissues by red blood cells is reflective of their ability to promote vasodilation and blood flow.”

Now, Stamler’s group has solidified the relationship between S-nitrosylation of hemoglobin in stored RBCs and the ability of those cells to deliver oxygen.

In mice, transfusion of re-nitrosylated, stored mouse RBCs maintained baseline muscle oxygenation levels, whereas transfusion of untreated, stored RBCs decreased muscle oxygenation levels.

In a rat model for hemorrhage, transfusion of re-nitrosylated, stored rat RBCs restored muscle oxygenation levels to baseline, whereas transfusion of untreated, stored RBCs did not.

In two sheep models for anemia, transfusion of re-nitrosylated, stored sheep RBCs increased muscle oxygenation and cardiovascular and renal function compared with transfusion of untreated, stored RBCs.

Results were published in the *Proceedings of the National Academy of Sciences*.

“Our work shows that re-nitrosylation has profound effects on the ability of stored red blood cells to improve blood flow, oxygen delivery to tissues and organs and organ function,” said Stamler, corresponding author on the paper. “Our data suggest that it really is oxygen delivery that goes awry as stored red blood cells age. The findings also raise the idea that nitrosylated blood may be used therapeutically, in conditions such as myocardial infarction, to improve blood flow.”

“The fact that they employed four different models is important

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as it decreases the possibility that the observed results were due to model-specific effects,” said John Roback, an associate professor in the Department of Pathology and Laboratory Medicine and director of the Center for Transfusion and Cellular Therapy at the **Emory University School of Medicine**.

Roback noted that he also found the suggestion to apply re-nitrosylation to all banked blood provocative. “Most practitioners consider fresh banked blood very efficacious, but the authors are arguing that since even the freshest blood from the blood bank has lost most of its S-nitrosohemoglobin, even these blood units could be made more effective by re-nitrosylation,” he told *SciBX*.

“Because banked red blood cells appear to become depleted of S-nitrosohemoglobin after just a few days in storage, the re-nitrosylation strategy will most likely need to be used in the hospital setting and applied just prior to transfusion,” added Daniel Kim-Shapiro, a professor in the Department of Physics and director of the Translational Science Center at **Wake Forest University**.

Affecting adverse events and efficacy

Stamler said his group is planning a clinical trial to obtain baseline measurements on how transfusions with stored RBCs change oxygen delivery and then determine how such changes correlate with transfusion-related adverse events. He said his group has received a grant from the **NIH** to carry out the trial. He declined to disclose the grant amount.

“Such information will be needed before we can properly assess the ability of re-nitrosylation therapy to improve oxygen delivery in transfusion recipients and whether use of re-nitrosylated red blood cells will have an impact on clinical outcomes,” Stamler told *SciBX*.

Patients who receive RBCs can develop severe transfusion-related complications that lead to MI, organ injury and organ failure,^{9,10} though the incidences are estimated to be below 0.1%.¹¹

Kim-Shapiro said it will be important to determine how re-nitrosylation affects the mechanical properties of RBCs and the chemical by-products that are generated from the re-nitrosylation procedure.

Other questions are how long the re-nitrosylation strategy takes, how durable it is and how long hemoglobin in treated RBCs stays nitrosylated, said Timothy McMahon, an associate professor of medicine at the **Duke University School of Medicine**. He was not involved in the current study.

Roback said cost considerations are especially significant in the blood transfusion setting, as it is the most common medical procedure

performed at many hospitals and the number-one line item on hospital expense reports.

“It would be difficult at this time for blood banks to consider implementing such a technology, as they have been focusing on ways to decrease expenses,” he told *SciBX*.

He noted that although the ethyl nitrite-based re-nitrosylation method used on sheep RBCs appears to be the most viable of the three methods to take into the clinical trials, it likely would add to the cost of a transfusion. Stamler’s group re-nitrosylated mouse RBCs with purified nitric oxide (NO), rat RBCs with S-nitrosocysteine and sheep RBCs with ethyl nitrite.

Roback said that clinical data showing that re-nitrosylated RBCs improve patient outcomes over untreated RBCs are going to be needed before hospitals and blood banks consider whether to adopt the approach.

Duke and Case Western have multiple issued and filed patents covering re-nitrosylation of blood. Licensing details are available from the Case Western Reserve University Technology Transfer Office.

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COMPANIES AND INSTITUTIONS MENTIONED

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Duke University School of Medicine, Durham, N.C.
Emory University School of Medicine, Atlanta, Ga.
National Institutes of Health, Bethesda, Md.
Wake Forest University, Winston-Salem, N.C.

GSK and UC make up

By Lev Osherovich, Senior Writer

GlaxoSmithKline plc and the University of California have reconciled their differences related to whether researchers at the 10-campus system can participate in GSK's Discovery Fast Track program. The University of California technology transfer offices will now vet applications for potentially sensitive disclosures before the pharma lays eyes on them.

GSK launched Discovery Fast Track in May to allow academic researchers to form collaborations with the pharma to test disease-related hypotheses.¹

Under the original terms of the precompetitive program, prospective participants were asked to submit brief, nonconfidential disclosures to the pharma about their scientific ideas and plans for collaboration with GSK.

The program hit a snag when the technology transfer office of the University of California, Los Angeles barred researchers at that campus from participating.² That policy soon was widened to cover the entire UC system.

Brendan Rauw, associate vice chancellor and executive director of entrepreneurship at the UCLA Office of Intellectual Property and Industry Sponsored Research, said there was concern about the potential for disclosure of confidential information and conflict with third-party rights to the researchers' discoveries.

In June, GSK conducted discussions with Rauw and representatives of the UC Office of General Counsel and came up with a fix.

Under the modified terms of participation in the Discovery Fast Track program, technology transfer offices will electronically monitor submissions and disclosures by researchers at their campuses. The goal

is to ensure nothing confidential is disclosed to the pharma.

"The opportunity to review applications beforehand was a critical part of the agreement," said Rauw. "We wanted to ensure that our staff was involved in the process. Our oversight is now required."

"We always intended for the investigators to engage their own technology transfer offices," said Pearl Huang, VP and global head of GSK's Discovery Academic Partnerships (DPAC) unit, which runs the Discovery Fast Track program. "We heard from multiple offices that they were concerned about ensuring their engagement. We're now able to make sure that happens."

One concern about UC's efforts to vet contacts between academia and industry is that slow turnaround by technology transfer offices can delay collaborative research.

Rauw said the amended terms of the program require technology transfer offices to review Discovery Fast Track applications "on a tight time frame."

According to the program's website, technology transfer offices will have five days to review applications after the submission deadline, which is July 19.

If the technology transfer office fails to sign off by the deadline, the applications will be discarded.

Going forward, GSK will use the IP vetting system it set up at UC's request for all participants in the Discovery Fast Track program, not just those at UC campuses.

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COMPANIES AND INSTITUTIONS MENTIONED

GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
University of California, Oakland, Calif.
University of California, Los Angeles, Calif.

"The opportunity to review applications beforehand was a critical part of the agreement. We wanted to ensure that our staff was involved in the process. Our oversight is now required."

—Brendan Rauw,
University of California,
Los Angeles

Depressing sphingolipids

By Lev Osherovich, Senior Writer

European researchers have obtained the most compelling evidence yet that targeting sphingolipid metabolism could help treat depression.¹ The team has shown that two known antidepressants inhibit sphingolipid metabolism and now is planning to screen for other inhibitors that also elicit antidepressive effects.

Previous work has shown that some serotonin-specific reuptake inhibitor (SSRI) antidepressants have an inhibitory effect on a key lipid processing enzyme called sphingomyelin phosphodiesterase 1 acid lysosomal (SMPD1; ASM).² SMPD1 is a membrane protein facing the lysosomal lumen that converts sphingomyelin into ceramide (see Figure 1, “Hitting ceramide for depression”). Ceramide diffuses out of the lysosome to other cellular membranes to modulate signaling pathways involved in cell growth, inflammation and intracellular responses to bacterial infection.

A team, co-led by the University of Erlangen-Nuremberg’s Johannes Kornhuber and the University of Duisburg-Essen’s Erich Gulbins, has now shown that inhibiting SMPD1 has effects on the nervous system that could influence depression.

Gulbins is professor of molecular biology and medicine at Duisburg-Essen, and Kornhuber is professor of psychiatry and psychotherapy at Erlangen-Nuremberg.

“What was known before is that antidepressants like fluoxetine and amitriptyline functionally inhibit SMPD1,” said Kornhuber. “These compounds enter the lysosome and cause the detachment of SMPD1 from the membrane, leading to its proteolytic degradation.” However, according to Kornhuber, no direct link between this mechanism of action and the antidepressant effect of the compounds had been shown until now.

The team thus set out to test whether some of the effects of widely used antidepressants could be a result of SMPD1 inhibition.

Ceramide’s touch

The researchers first observed that cultured human neurons treated with the generic SSRI antidepressants fluoxetine and amitriptyline had lower *in vitro* SMPD1 activity and ceramide levels than untreated neurons.

Eli Lilly and Co. markets fluoxetine as Prozac, whereas amitriptyline is no longer marketed as an antidepressant because of safety issues. Both compounds are suspected to have additional mechanisms of action on top of their SSRI activity.

The team then engineered mice with altered levels of *Smpd1* to test the enzyme’s effect on the nervous system. *Smpd1* knockouts had lower ceramide levels and *Smpd1* overexpressing mice had higher ceramide levels than wild-type controls.

Treatment with fluoxetine or amitriptyline reduced ceramide levels in *Smpd1*-overexpressing mice but did not alter ceramide levels in *Smpd1* knockouts, suggesting *Smpd1* was needed to mediate the drugs’ effects on ceramide.

The group then examined the effects of *Smpd1* activity on neurogenesis and neuronal survival, which are compromised in animal models for

depression. In a mouse model for stress, animals overexpressing *Smpd1* had lower levels of hippocampal neurogenesis and neuronal survival than wild-type controls.

Antidepressants improved neurogenesis and neuronal survival in wild-type and *Smpd1*-overexpressing mice but not in *Smpd1* knockouts.

In addition, *Smpd1* activity correlated with depressive behavior in mice. In assays of stress-induced depression, antidepressants had little effect on mice lacking *Smpd1* but alleviated depression-associated behavior in wild-type controls and *Smpd1*-overexpressing animals.

Finally, the team directly injected ceramide into the hippocampus of wild-type mice and saw an increase in depression-like behavior compared with what was seen in vehicle-treated controls.

“We hypothesized that high levels of ceramide were needed to produce depressive behavior,” said Kornhuber. “When we overexpressed *Smpd1*, these mice showed spontaneous depressive behavior. When we injected ceramide into the hippocampus of mice, this also generated a depressed mood.”

Results were reported in *Nature Medicine*.

Getting selective

Overall, the findings suggest high ceramide levels contribute to cellular and behavioral correlates of depression. The findings also argue that

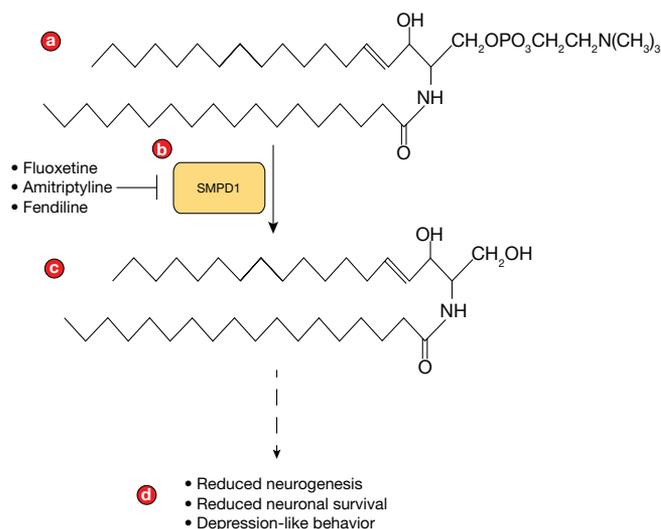


Figure 1. Hitting ceramide for depression. Gulbins *et al.* have evidence that blocking the production of ceramide, a phospholipid signaling molecule, has antidepressive effects.

Ordinarily, the membrane-associated phospholipid sphingomyelin [a] is cleaved by sphingomyelin phosphodiesterase 1 acid lysosomal (SMPD1; ASM) [b] to yield ceramide [c]. Gulbins *et al.* showed that in cell culture and mice, accumulation of ceramide correlated with depressive behavior and reduced neuronal growth and survival [d].

The team also showed that two known antidepressants—fluoxetine and amitriptyline—lowered ceramide levels and decreased activity of SMPD1 compared with no treatment. Fendiline, a nonselective calcium channel blocker, also reduced SMPD1 activity and normalized depression-like behavior.

commonly used antidepressants have an additional mechanism of action—inhibition of SMPD1—that is distinct from the inhibition of serotonin reuptake.

From a drug discovery standpoint, the results open up the possibility of directly targeting SMPD1 or other enzymes that affect ceramide levels to treat depression.

In their *Nature Medicine* paper, Kornhuber and Gulbins also reported preliminary efforts to identify small molecules that modulate ceramide levels and depression in mice.

The team screened a panel of 250 neurologically active compounds for effects on ceramide levels in cell culture and found other molecules that acted like fluoxetine and amitriptyline. Among these was fendiline, a calcium channel blocker that had not previously been tested as an antidepressant. In mouse models for depression, fendiline mimicked the effects of fluoxetine and amitriptyline.

Kornhuber cautioned that fluoxetine, amitriptyline and fendiline are likely to have additional biochemical effects beyond inhibiting SMPD1 that contribute to their antidepressive effects.

“We believe that lowering ceramide is an important mechanism of antidepressant action,” said Kornhuber. “Does every antidepressant drug work this way? Probably not; but many antidepressants do.” He noted that other SSRIs tested by the team had antidepressive effects but did not alter ceramide levels.

“We believe that lowering ceramide is an important mechanism of antidepressant action. Does every antidepressant drug work this way? Probably not; but many antidepressants do.”

—Johannes Kornhuber,
University of Erlangen-
Nuremberg

The next step is to identify selective SMPD1 inhibitors and test their effect on hippocampal ceramide levels and depressive behavior in mice.

Kornhuber further noted that complete inhibition of SMPD1 could have undesired consequences.

“If you have low levels of ceramide, it’s bad for the central nervous system,” said Kornhuber, noting that a genetic defect in ceramide synthesis causes certain forms of Niemann-Pick disease. “But if you lower SMPD1 levels by as much as 80%, it’s well tolerated.”

What remains unclear is whether excess intracellular ceramide leads to depression in humans. Kornhuber and Gulbins found that changes in ceramide levels caused by tinkering with *Smpd1* expression led to changes in the activity of protein kinase B (PKB; PKBA; AKT; AKT1), a kinase that participates in a broad range of intracellular signaling pathways.

Uncovering the downstream mechanisms of ceramide signaling in depression will require further cell culture and *in vivo* studies.

The findings described in the paper have not been patented.

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COMPANIES AND INSTITUTIONS MENTIONED

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Cancer genome statistics lesson

By Chris Cain, Senior Writer

The **Broad Institute of MIT and Harvard** has upgraded its original algorithm for cancer genome analysis to account for gene-specific differences in mutation rates.¹ Application of the improved algorithm could help identify previously overlooked mutations, and in one type of cancer it already allowed the researchers to narrow down the number of associated mutations.

Numerous large-scale cancer genome sequencing projects launched in the past decade are beginning to generate a wealth of genetic data on a growing number of tumor types. Interpretation of these data has required the development of new statistical approaches to help identify somatic mutations in tumors that are significantly associated with disease.

Several groups led by the Broad Institute have developed algorithms that account for factors such as genomewide background mutation rates, which vary from cancer to cancer, and may determine associations between genes and disease.

However, potential issues with the accuracy of these models, which may go unnoticed when applied to small data sets, can become amplified when analyzing large data sets.

“We knew there was a problem several years ago, but it didn’t manifest that severely,” Gad Getz, director of cancer genome computational analysis at the Broad Institute, told *SciBX*. “It became an acute problem as larger data sets were generated. When you have a small data set, for a mutation to be considered significant it must have a signal much higher than background. As sample sizes increase, you get the statistical power to see smaller variations compared with the background model, and if you have an inaccurate model, this becomes a real problem.”

Thus, Getz and his team set out to build a more accurate algorithm for large-scale cancer genome analysis.

First, the team built a data set to inform the model that integrated whole-genome and whole-exome sequences from about 3,000 patient tumor samples, along with matched data from the patients’ healthy tissue. About 92% of the data were collected at the Broad Institute.

The team first quantified the background mutation rate for 27 sequenced cancer types, then quantified the spectrum of mutations and found that they varied by cancer type in predictable ways. For example, melanomas were more prone to cytosine-to-thymidine mutations, which are caused by misrepair of UV-damaged base pairs.

Most importantly, the group used the sequencing information to determine regional differences in mutation rates across the genome, enabling the analysis and quantification of gene-specific rates of mutation.

Interestingly, the levels of individual genes inversely correlated with their mutation rates, meaning genes with low expression were more frequently mutated. The average mutation rate for the 25% of genes with the lowest expression was roughly 3-fold higher than that for the 25% of genes with the highest expression. Although the link between gene levels and mutation rates had been previously shown, this analysis represents

the most extensive quantification to date of the effects of transcription on mutation rate.

More unexpected was the finding that gene-specific mutation rates also varied based on the replication timing of a given gene during the cell cycle. The latest 10% of genes replicated had about a 2-fold higher mutation rate than the earliest 10% of genes replicated.

The authors used the new insights to update the Broad Institute’s algorithm for cancer genome analysis, dubbed Mutational Significance (MutSig). The key improvement in the updated version, called MutSig Covariate (MutSigCV), is that it uses gene expression and replication timing—which co-vary with mutation rate—to estimate gene-specific mutation rates.

When applied to a recently published analysis of squamous cell lung cancer genome sequences conducted by members of Getz’s team as part of **The Cancer Genome Atlas** (TCGA) project,² MutSigCV narrowed down the number of mutated genes significantly associated with the cancer from 450 to 11.

Results were published in *Nature*.

Writing in *Nature*, Getz and his team said inaccuracies in large-scale analysis likely have caused numerous false-positive results to show up in the literature. “The expectation has been that larger sample sizes will increase the power both to detect true cancer driver genes (sensitivity) and to distinguish them from the background of random mutations (specificity).” But “recent results seem to show the opposite phenomenon: with large sample sizes, the list of apparently significant cancer-associated genes grows rapidly and implausibly,” according to the authors.

Indeed, the data in the paper show that cancer-associated genes such as olfactory genes, large genes and others, described as “highly suspicious” by the authors, had low expression and replicated late, suggesting they are prone to higher mutation rates. Thus, said the authors, mutations in these genes may not be truly associated with cancer more frequently than chance alone, contrary to predictions by algorithms that did not take gene-specific mutation rates into account.

“This is a major advance, and all groups need to consider these ideas and these tools—and they are already doing this,” said Getz, who noted that MutSigCV is now the routine algorithm used for analysis by TCGA.

Jun Wang, director of **BGI**, said similar efforts are under way at his institute.

“BGI has already accumulated a huge amount of cancer-omics data, and we are also considering optimizing the algorithm of detecting putative cancer driver genes. There is no doubt that this paper makes a good start for this kind of effort,” said Wang.

Collections catalog

Although advances in cancer genome analysis will undoubtedly improve the quality of the catalog of cancer-associated mutations,

“Previously the model was using a constant rate of mutation along the genome; now we have a variable rate. This tool could resurrect genes that would not be caught with a naïve model because significant changes in genes with mutation rates lower than average could be better detected.”

—Gad Getz,
Broad Institute of MIT
and Harvard

it may have little practical effect on the industry's pursuit of cancer targets.

"The described algorithm will aid defining cancer-causing gene candidates and distinguishing them from passenger genes. Unfortunately, most cancer genome sequencing efforts remain more or less sophisticated counting exercises that contribute little to the identification of driver genes or genes important for understanding—or treating—cancer. Functional studies cannot be replaced with mathematical analyses," said Christoph Lengauer, CSO of **Blueprint Medicines**.

Blueprint is developing selective kinase inhibitors targeting cancers driven by genomic alterations.

Markus Warmuth, president and CEO of **H3 Biomedicine Inc.**, said the new algorithm represents a major advance but cautioned there is a trade-off between being too stringent about statistical cutoffs and missing possible cancer-associated genes on the one hand and being too lax and generating catalogs of false positives on the other.

"To some degree, it depends on what you want to accomplish with the data. If you are at a major medical center, and you have 25 targeted therapies in your repertoire, you only want actionable mutations and you want to be as stringent as possible. However, in the research side or when looking at candidates for drug discovery, you may want to be a little less stringent to cast a wider net, and to follow up with functional studies to rule out false positives," he said.

Getz emphasized that the new algorithm should not be viewed solely as being more stringent than prior analysis methods. "We are not just ruling out genes with this method; it also has the potential to find more genes. Previously the model was using a constant rate of mutation along the genome; now we have a variable rate. This tool could resurrect genes that would not be caught with a naïve model because significant changes in genes with mutation rates lower than average could be better detected."

Getz said his team will continue to improve upon MutSigCV as additional data are generated, and whole-genome sequencing information will be of particular importance. In this study, 2,957 whole-exome data sets were analyzed, compared with only 126 whole-genome data sets.

Yong Hou, director of the cancer research division of the BGI, agreed that increased access to whole-genome data sets is key. "The continuous optimizing of the algorithm for detecting putative cancer genes, accompanied by the accumulating of more and more cancer-omics data, especially on the whole-genome level, is still a major task for cancer genomics research," he said.

The Broad Institute has filed for patents covering MutSigCV. The algorithm is freely available for not-for-profit use and will be available for licensing to for-profit organizations.

Cain, C. *SciBX* 6(27); doi:10.1038/scibx.2013.676

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2. The Cancer Genome Atlas Research Network. *Nature* **489**, 519–525 (2012)

COMPANIES AND INSTITUTIONS MENTIONED

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Blueprint Medicines, Cambridge, Mass.
Broad Institute of MIT and Harvard, Cambridge, Mass.
The Cancer Genome Atlas, Bethesda, Md.
H3 Biomedicine Inc., Cambridge, Mass.



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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				
Breast cancer	Chemokine CX3C motif ligand 1 (CX3CL1; fractalkine); HER2 (EGFR2; ErbB2; neu)	<p>Mouse studies suggest inhibiting CX3CL1 could help treat HER2⁺ breast cancers. In a transgenic mouse model for Her2⁺ breast cancer, adenovirus-mediated overexpression of Cx3cl1 increased the number of mammary tumors compared with normal expression. In the mouse model, <i>Cx3cl1</i> knockout delayed tumorigenesis and decreased the number of Her2-driven mammary tumors compared with no knockout. Next steps could include identifying pharmacological inhibitors of CX3CL1.</p> <p>SciBX 6(27); doi:10.1038/scibx.2013.677 Published online July 18, 2013</p>	Patent and licensing status unavailable	<p>Tardaguila, M. <i>et al. Cancer Res.</i>; published online May 29, 2013; doi:10.1158/0008-5472.CAN-12-3828 Contact: Santos Mañes, National Center for Biotechnology Information, Madrid, Spain e-mail: smanes@cnb.csic.es</p>
Breast cancer	Toll-like receptor 7 (TLR7)	<p>Mouse studies suggest stimulating TLR7 on plasmacytoid dendritic cells (pDCs) could help treat breast cancer. In an immune-competent breast cancer mouse model, a TLR7 ligand or the TLR7 agonist SM360320 activated pDCs and caused tumor regression, whereas vehicle did not. The mice also were protected from subsequent challenges with murine mammary tumor cells for at least three months after pDC stimulation. Next steps could include analyzing the immunological effects resulting from pDC activation.</p> <p>SM360320 is a research reagent. Meda AB and Valeant Pharmaceuticals International Inc. market the TLR7-targeting immune response modulator Aldara imiquimod to treat basal cell carcinoma (BCC).</p> <p>Telormedix S.A.'s TLR7 agonist TMX-102 is in Phase II testing to treat bladder cancer. The company's TLR7 agonist TMX-202 is in preclinical development for skin and bladder cancers.</p> <p>Idera Pharmaceuticals Inc.'s IMO-4200, a dual agonist of TLR7 and TLR8, is in preclinical development for lymphoma.</p> <p>SciBX 6(27); doi:10.1038/scibx.2013.678 Published online July 18, 2013</p>	Patent and licensing status unavailable	<p>Le Mercier, I. <i>et al. Cancer Res.</i>; published online May 30, 2013; doi:10.1158/0008-5472.CAN-12-3058 Contact: Nadege Goutagny, Cancer Research Center of Lyon, University of Lyon, Lyon, France e-mail: nadege.goutagny@lyon.unicancer.fr</p>
Chronic myelogenous leukemia (CML)	BCR-ABL tyrosine kinase	<p><i>In vitro</i> and mouse studies identified a pyrazolo[3,4-<i>d</i>]pyrimidine that could help treat CML with the T315I resistance mutation in BCR-ABL. In mouse myeloid cells that express T315I mutant Bcr-Abl, compared with cells expressing wild-type Bcr-Abl, the molecule showed increased cytotoxicity. In mice grafted with myeloid cells that expressed T315I mutant Bcr-Abl, the inhibitor decreased tumor volume by more than 50% compared with vehicle. Next steps could include testing the inhibitor in additional animal models for CML.</p> <p>SciBX 6(27); doi:10.1038/scibx.2013.679 Published online July 18, 2013</p>	Patent and licensing status unavailable	<p>Radi, M. <i>et al. J. Med. Chem.</i>; published online June 7, 2013; doi:10.1021/jm400233w Contact: Maurizio Botta, University of Siena, Siena, Italy e-mail: botta.maurizio@gmail.com Contact: Silvia Schenone, University of Genoa, Genoa, Italy e-mail: schensil@unige.it</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	V-set domain containing T cell activation inhibitor 1 (VTCN1; B7-H4)	Studies <i>in vitro</i> , in mice and in patient samples identified anti-B7-H4 single-chain variable antibody fragments (scFvs) that could help treat cancer. In ovarian cancer samples from 15 patients, B7-H4 was expressed on the surface of all samples. A screen in yeast identified scFvs that bound B7-H4, and these scFvs reversed B7-H4-mediated inhibition of donor T cell activation. In a humanized mouse model for ovarian cancer, scFvs against B7-H4 reduced tumor growth. Next steps could include testing anti-B7-H4 scFvs or antibodies in additional ovarian cancer models. SciBX 6(27); doi:10.1038/scibx.2013.680 Published online July 18, 2013	Patent and licensing status unavailable	Dangaj, D. <i>et al. Cancer Res.</i> ; published online May 30, 2013; doi:10.1158/0008-5472.CAN-12-3457 Contact: Nathalie Scholler, SRI International, Menlo Park, Calif. e-mail: nathaliescholler@gmail.com
Liver cancer	γ -Secretase; notch 1 (NOTCH1)	Cell culture and mouse studies suggest antagonizing NOTCH1 could be useful for treating cholangiocellular carcinoma (CCC), a form of liver cancer. Human cell lines derived from CCC tumors and CCC tissues had high levels of NOTCH1 signaling compared with other tumor lines and normal tissue. In mice, transgenic expression of a constitutively active form of NOTCH1 led to CCC. Also in mice, a γ -secretase inhibitor blocked activation of NOTCH1 and decreased CCC growth compared with vehicle. Next steps include further characterization of NOTCH1 activity in human CCC tumors and testing anti-NOTCH1 mAbs in mouse models. At least six companies have NOTCH1 mAbs or γ -secretase inhibitors in preclinical or Phase I testing to treat various cancers. SciBX 6(27); doi:10.1038/scibx.2013.681 Published online July 18, 2013	Unpatented; licensing status not applicable	Zender, S. <i>et al. Cancer Cell</i> ; published online May 30, 2013; doi:10.1016/j.ccr.2013.04.019 Contact: Nisar P. Malek, Eberhard Karls University of Tuebingen, Tuebingen, Germany e-mail: nisar.malek@med.uni-tuebingen.de
Solid tumors	TNF-like weak inducer of apoptosis receptor (TNFRSF12A; TWEAKR; FN14)	Mouse and cell culture studies identified an antibody-drug conjugate against FN14 that could help treat FN14 ⁺ solid tumors. In a panel of five FN14 ⁺ human breast cancer cell lines, a humanized, dimeric, single-chain anti-FN14 antibody fused to recombinant gelonin toxin was cytotoxic and had IC ₅₀ values of 0.1–2.4 nM. In a mouse xenograft model for FN14 ⁺ human breast cancer, the FN14-targeted immunotoxin significantly decreased tumor growth ($p < 0.01$) and increased survival ($p < 0.0001$) compared with saline. Next steps could include testing the targeted immunotoxin in other FN14 ⁺ solid tumors. SciBX 6(27); doi:10.1038/scibx.2013.682 Published online July 18, 2013	Patent and licensing status unavailable	Zhou, H. <i>et al. Cancer Res.</i> ; published online May 30, 2013; doi:10.1158/0008-5472.CAN-13-0187 Contact: Michael G. Rosenblum, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: mrosenbl@mdanderson.org

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Endocrine/metabolic disease				
Diabetes	Glucagon (GCG); glucagon receptor (GCGR)	<p>Cell culture and mouse studies suggest an aptamer that binds to GCG could help treat diabetes. <i>In vitro</i> selection and chemical optimization yielded a DNA aptamer, NOX-G15, that bound GCG with a K_d of 3 nM. In epithelial cells overexpressing human GCGR, the aptamer inhibited GCG signaling. In nongenetic mouse models for type 1 and type 2 diabetes, the aptamer decreased peak blood glucose levels in glucose tolerance tests compared with vehicle but did not affect fasting blood glucose levels. Next steps include testing the aptamer and related molecules in additional mouse models for type 1 diabetes.</p> <p>Noxxon Pharma AG's NOX-G15 and NOX-G16 are in preclinical development for diabetes.</p> <p>Novo Nordisk A/S and Paladin Labs Inc. market GlucaGen, a recombinant form of glucagon, to treat diabetes.</p> <p>At least seven other companies have GCG or GCGR antagonists in Phase III or earlier testing to treat diabetes or obesity.</p> <p>SciBX 6(27); doi:10.1038/scibx.2013.683 Published online July 18, 2013</p>	<p>Patent application filed covering several GCG-binding aptamers; available for licensing from Noxxon Pharma</p> <p>Contact: Aram Mangasarian, Noxxon Pharma AG, Berlin, Germany e-mail: amangasarian@noxxon.com</p>	<p>Vater, A. <i>et al. J. Biol. Chem.</i>; published online June 6, 2013; doi:10.1074/jbc.M112.444414</p> <p>Contact: Sven Klussmann, Noxxon Pharma AG, Berlin, Germany e-mail: sklussmann@noxxon.com</p>
Hematology				
Transfusion	Hemoglobin	<p>Rodent and sheep studies suggest S-nitrosylation of hemoglobin in banked red blood cells (RBCs) could improve blood transfusion outcomes. In a rat model for hemorrhage, transfusion of re-nitrosylated, stored RBCs restored muscle oxygenation levels to baseline, whereas transfusion of untreated, stored RBCs did not. In two sheep models of anemia, transfusion of re-nitrosylated, stored RBCs increased muscle oxygenation compared with transfusion of untreated, stored RBCs and also improved cardiovascular and renal function. Next steps include characterizing baseline oxygenation levels in humans after receiving banked blood and comparing re-nitrosylated blood with banked blood in a clinical trial (<i>see S-nitrosylation boon to blood transfusions, page 1</i>).</p> <p>SciBX 6(27); doi:10.1038/scibx.2013.684 Published online July 18, 2013</p>	<p>Multiple patent applications filed covering re-nitrosylation of blood; licensing details available from Case Western Reserve University</p>	<p>Reynolds, J.D. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online June 24, 2013; doi:10.1073/pnas.1306489110</p> <p>Contact: Jonathan S. Stamler, Case Western Reserve University, Cleveland, Ohio e-mail: jonathan.stamler@case.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Infectious disease				
Crohn's disease	Bacterial fimbrial adhesin (fimH)	Cell culture and <i>ex vivo</i> studies suggest thiazolylaminomannosides could help treat Crohn's disease. Adherent-invasive <i>Escherichia coli</i> (AIEC), which are associated with Crohn's disease, use fimH to bind to oligomannosides on the surface of intestinal epithelial cells, leading to inflammation. In cultured human intestinal cells expressing the fimH receptor carcinoembryonic antigen related cell adhesion molecule 6 (CEACAM6; NCA; CD66c), a thiazole-modified <i>N</i> -mannoside decreased AIEC attachment compared with no treatment. In colonic explants from a mouse model for AIEC colonization, the thiazole-modified <i>N</i> -mannoside inhibited AIEC from binding to the mucosal surface with 10- to 100-fold higher potency than previously published <i>D</i> -mannosides. Next steps include testing the specificity of <i>N</i> -mannosides to different pathogenic fimH variants and determining <i>in vivo</i> treatment efficacy in a mouse model to assess preventive and curative effects. SciBX 6(27); doi:10.1038/scibx.2013.685 Published online July 18, 2013	Patent application filed; available for licensing from France Scientific Innovation and Transfer SA	Brument, S. <i>et al. J. Med. Chem.</i> ; published online June 12, 2013; doi:10.1021/jm400723n Contact: Sébastien G. Gouin, University of Nantes Angers Le Mans (L'UNAM), Nantes, France e-mail: sebastien.gouin@univ-nantes.fr Contact: Rostyslav O. Bilyy, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine e-mail: r.bilyy@gmail.com Contact: Arlette Darfeuille-Michaud, French National Institute for Agricultural Research (INRA), Clermont-Ferrand, France e-mail: arlette.darfeuille-Michaud@u-clermont1.fr Contact: Julie Bouckaert, Lille 1 University, Lille, France e-mail: julie.bouckaert@univ-lille1.fr
HCV	β -Site APP-cleaving enzyme 1 (BACE1)	Cell culture studies suggest inhibiting the interaction between HCV and transferrin receptor could help prevent infection. In human hepatocarcinoma cells, small interfering RNA-, antibody- or small molecule-mediated inhibition of transferrin receptor decreased viral infection compared with no inhibition. In the cells, an antibody against the transferrin receptor decreased infection by engineered HCV particles compared with other engineered viral particles. Next steps include mapping the interaction between the transferrin receptor and HCV. Roche has an anti-BACE1 and anti-transferrin receptor antibody in preclinical testing to treat Alzheimer's disease (AD). SciBX 6(27); doi:10.1038/scibx.2013.686 Published online July 18, 2013	Unpatented; licensing status not applicable	Martin, D.N. & Uprichard, S.L. <i>Proc. Natl. Acad. Sci. USA</i> ; published online June 10, 2013; doi:10.1073/pnas.1301764110 Contact: Susan L. Uprichard, Loyola University Medical Center, Maywood, Ill. e-mail: suprichard@lumc.edu
HIV/AIDS	Protein kinase C δ (PRKCD)	<i>In vitro</i> studies suggest analogs of prostratin, a PRKCD inhibitor, could help treat HIV infection. Chemical synthesis and <i>in vitro</i> testing of prostratin analogs identified multiple compounds that inhibited PRKCD with 10- to 100-fold greater potency than prostratin. In a human cell-based assay, the compounds reactivated latent HIV at nanomolar EC ₅₀ values, whereas prostratin reactivated the virus at a micromolar EC ₅₀ value. In CD4 ⁺ T cells from patients infected with HIV who were on highly active antiretroviral therapy, a 50 nM dose of the lead compound increased HIV RNA production by >100-fold compared with the same dose of prostratin. Ongoing work includes testing the toxicity of the analogs in normal mice. The AIDS Research Alliance has prostratin in preclinical testing to treat HIV infection. SciBX 6(27); doi:10.1038/scibx.2013.687 Published online July 18, 2013	Patented by Stanford University; licensing status undisclosed	Beans, E.J. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 28, 2013; doi:10.1073/pnas.1302634110 Contact: Paul A. Wender, Stanford University, Stanford, Calif. e-mail: wenderp@stanford.edu Contact: Jerome A. Zack, University of California, Los Angeles, Calif. e-mail: jzack@ucla.edu Contact: Tae-Wook Chun, National Institutes of Health, Bethesda, Md. e-mail: twchun@nih.gov

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
<i>Staphylococcus</i>	<i>Staphylococcus</i> leukotoxin AB (lukAB); complement receptor 3 (CR3; CD11b); MAC-1 (CD11b-CD18)	Cell culture studies suggest inhibiting lukAB signaling could help treat <i>Staphylococcus aureus</i> infection. In cultured, neutrophil-like cells, immunoprecipitation experiments showed that lukAB directly bound the CD11b subunit of MAC-1. In these cells, small hairpin RNA knockdown of CD11b or pretreatment with an anti-CD11b antibody decreased <i>S. aureus</i> -mediated membrane damage compared with no knockdown or pretreatment. Next steps include developing additional tools to evaluate the contribution of lukAB to <i>S. aureus</i> pathogenicity during infections. SciBX 6(27); doi:10.1038/scibx.2013.688 Published online July 18, 2013	Patent application filed; licensed to an undisclosed company	DuMont, A.L. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 10, 2013; doi:10.1073/pnas.1305121110 Contact: Victor J. Torres, New York University School of Medicine, New York, N.Y. e-mail: victor.torres@nyumc.org
Neurology				
Alzheimer's disease (AD)	Cyclin dependent kinase 5 (CDK5); glycogen synthase kinase 3 β (GSK3B)	Mouse studies suggest dual diaminothiazole CDK5 and GSK3B inhibitors could help treat AD. Diaminothiazole compounds inhibited the microtubule-associated protein- τ (MAPT; TAU; FTDP-17)-phosphorylating kinases CDK5 and GSK3B with low nanomolar potencies. In two mouse models for AD, the lead inhibitor decreased TAU phosphorylation and insoluble TAU levels compared with vehicle. The lead inhibitor also increased neuron density and improved memory. Next steps include screening and designing additional small molecule inhibitors of CDK5 and GSK3B. Neurim Pharmaceuticals Ltd.'s GSK3B inhibitor, Neu-120, is in Phase II testing to treat Parkinson's disease (PD). Eli Lilly and Co.'s GSK3B inhibitor, LY2090314, is in Phase II trials to treat pancreatic cancer and leukemia. DiaMedica Inc.'s DM-99, a GSK3B inhibitor, is in Phase II testing to treat diabetes. SciBX 6(27); doi:10.1038/scibx.2013.689 Published online July 18, 2013	Unpatented; compounds available for licensing	Zhang, X. <i>et al. J. Biol. Chem.</i> ; published online June 4, 2013; doi:10.1074/jbc.M112.436402 Contact: Kenneth S. Kosik, University of California, Santa Barbara, Calif. e-mail: kenneth.kosik@lifesci.ucsb.edu
Depression	Sphingomyelin phosphodiesterase 1 acid lysosomal (SMPD1; ASM)	Mouse and cell culture studies suggest inhibiting SMPD1 could be useful for treating depression. In human neuronal cell lines and in mice, the antidepressants amitriptyline and fluoxetine decreased SMPD1 activity and ceramide levels compared with vehicle. In a mouse model for stress-induced depression, the SMPD1 inhibitor fendiline decreased depression-like behaviors with potency comparable to that of antidepressant drugs. Next steps include identifying potent, selective inhibitors of SMPD1 and characterizing their effects in mouse models for depression. Eli Lilly and Co. markets Prozac fluoxetine to treat major depressive disorder, obsessive-compulsive disorder (OCD), bulimia nervosa and panic disorder. Amitriptyline is a generic antidepressant. Fendiline is a research reagent (<i>see</i> Depressing sphingolipids , page 5). SciBX 6(27); doi:10.1038/scibx.2013.690 Published online July 18, 2013	Unpatented; licensing status not applicable	Gulbins, E. <i>et al. Nat. Med.</i> ; published online June 16, 2013; doi:10.1038/nm.3214 Contact: Erich Gulbins, University of Duisburg-Essen, Essen, Germany e-mail: erich.gulbins@uni-due.de Contact: Johannes Kornhuber, University of Erlangen-Nuremberg, Erlangen, Germany e-mail: johannes.kornhuber@uk-erlangen.de

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Multiple sclerosis (MS)	Mannosyl (α -1,6-)-glycoprotein β -1,6- <i>N</i> -acetylglucosaminyltransferase isozyme B (MGAT5B)	Mouse studies suggest inhibiting MGAT5B could help treat MS. In a mouse model for chemically induced demyelination, <i>MGAT5B</i> knockout increased remyelination compared with no knockout. Next steps could include identifying small molecules that inhibit MGAT5B. SciBX 6(27); doi:10.1038/scibx.2013.691 Published online July 18, 2013	Patent and licensing status unavailable	Kanekiyo, K. <i>et al. J. Neurosci.</i> ; published online June 12, 2013; doi:10.1523/JNEUROSCI.3137-12.2013 Contact: Naoyuki Taniguchi, RIKEN, Saitama, Japan e-mail: tani52@wd5.so-net.ne.jp
Seizures	Calcium channel voltage-dependent L type- α 1C subunit (CACNA1C; Cav1.2)	Rodent and cell culture studies suggest inhibiting Cav1.2 could help treat febrile seizures. In rat hippocampal pyramidal neurons, Cav1.2 was associated with temperature-induced, seizure-related firing activity. In rat pups, the nonselective calcium channel blocker nimodipine decreased the incidence and duration of febrile seizures compared with vehicle. Next steps could include screening for Cav1.2-selective inhibitors and evaluating their effect in models for febrile seizure. Nimodipine is a generic drug used to improve neurological outcomes after subarachnoid hemorrhage. Oral formulations of the drug are marketed by Bayer AG as Nimotop and Arbor Pharmaceuticals Inc. as Nymalize. SciBX 6(27); doi:10.1038/scibx.2013.692 Published online July 18, 2013	Patent and licensing status unavailable	Radzicki, D. <i>et al. J. Neurosci.</i> ; published online June 12, 2013; doi:10.1523/JNEUROSCI.5482-12.2013 Contact: Marco Martina, Northwestern University, Chicago, Ill. e-mail: m-martina@northwestern.edu
Renal disease				
Renal failure	Nitric oxide (NO); γ -glutamyltransferase (GGT)	<i>Ex vivo</i> tissue studies suggest NO-generating prodrugs could help treat acute renal failure. In <i>ex vivo</i> rat aortic ring tissue, a lead <i>N</i> -hydroxyguanidine analog released NO and induced vasodilation. In the aortic ring tissue, a glutamyl prodrug of the lead compound and the renal-specific enzyme GGT increased NO release and vasodilation compared with the prodrug alone. In isolated, perfused rat kidneys, the prodrug increased vasodilation compared with the unconjugated lead compound or an inactive control compound. Next steps include testing the prodrug in models for ischemia- and sepsis-induced acute renal failure. SciBX 6(27); doi:10.1038/scibx.2013.693 Published online July 18, 2013	Unpatented; available for partnering	Zhang, Q. <i>et al. J. Med. Chem.</i> ; published online June 5, 2013; doi:10.1021/jm400146r Contact: Ian L. Megson, The University of The Highlands and Islands, Inverness, U.K. e-mail: ian.megson@uhi.ac.uk
Various				
Atherosclerosis; hyperlipidemia	MicroRNA-30c (miR-30c)	Mouse and cell culture studies suggest miR-30c could help treat hyperlipidemia and atherosclerosis. In mice fed a Western diet, hepatic overexpression of miR-30c decreased plasma lipid concentrations and hepatic lipoprotein production compared with overexpression of scrambled miRNA. In a mouse model for atherosclerosis, overexpression of miR-30c decreased the number and size of atherosclerotic plaques compared with overexpression of scrambled miRNA. Next steps include evaluating miR-30c in additional animal models. SciBX 6(27); doi:10.1038/scibx.2013.694 Published online July 18, 2013	Patent pending; available for licensing from SUNY Downstate Medical Center Contact: David Schoenhaut, SUNY Downstate Medical Center, Brooklyn, N.Y. e-mail: david.schoenhaut@downstate.edu	Soh, J. <i>et al. Nat. Med.</i> ; published online June 9, 2013; doi:10.1038/nm.3200 Contact: M. Mahmood Hussain, SUNY Downstate Medical Center, Brooklyn, N.Y. e-mail: mhussain@downstate.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer; inflammation	E selectin (SELE; CD62E)	<p><i>In vitro</i> studies suggest nitroindole-triazole analogs of a tetrasaccharide sialyl Lewis^x (sLe^x) mimic could help treat cancer and inflammatory diseases. sLe^x is the minimum carbohydrate epitope required for inhibitory binding to SELE, which has been linked to cancer and inflammatory diseases. A series of <i>in vitro</i> assays identified nitroindole-triazole analogs of an sLe^x mimic that bound SELE with low nanomolar affinities. In these assays, the lead analogs also showed >200-fold longer SELE binding half-lives compared with the unmodified mimic. Next steps could include testing the compounds in animal models for cancer and inflammatory disease.</p> <p>GlycoMimetics Inc. and Pfizer Inc. have GMI-1070, a glycomimetic inhibitor of SELE, SELP (CD62P) and SELL (CD62L), in Phase II trials to treat sickle cell disease.</p> <p>GlycoMimetics' SELE inhibitor GMI-1271 is in preclinical testing to treat acute myelogenous leukemia (AML) and thrombosis.</p> <p>SciBX 6(27); doi:10.1038/scibx.2013.695 Published online July 18, 2013</p>	Patented by the University of Basel; licensed to GlycoMimetics	Egger, J. <i>et al. J. Am. Chem. Soc.</i> ; published online June 6, 2013; doi:10.1021/ja4029582 Contact: Beat Ernst, University of Basel, Basel, Switzerland e-mail: beat.ernst@unibas.ch
Obesity; obsessive compulsive disorder (OCD)	Discs large homolog- associated protein 3 (DLGAP3; SAPAP3); melanocortin 4 receptor (MC4R);	<p>Mouse studies suggest inhibition of MC4R could help treat OCD and inhibition of SAPAP3 could help treat obesity. In a <i>Sapap3</i> knockout mouse model for OCD, <i>Mc4r</i> knockout or intracerebroventricular infusion of a small molecule MC4R antagonist normalized compulsive grooming behaviors. In an <i>Mc4r</i> knockout mouse model for obesity, <i>Sapap3</i> knockout reversed excessive weight gain and metabolic markers of obesity. Next steps include dissecting downstream pathways of MC4R and SAPAP3 using the same mouse models and determining whether the results extend to other OCD and obesity models.</p> <p>SciBX 6(27); doi:10.1038/scibx.2013.696 Published online July 18, 2013</p>	Unpatented; licensing status not applicable	Xu, P. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 10, 2013; doi:10.1073/pnas.1308195110 Contact: Andrew A. Pieper, The University of Iowa, Iowa City, Iowa e-mail: andrew-pieper@uiowa.edu Contact: Michael Lutter, same affiliation as above e-mail: michael-lutter@uiowa.edu Contact: Robert C. Malenka, Stanford University, Stanford, Calif. e-mail: malenka@stanford.edu



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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			
Fluorescence-based bilirubin assay	A fluorescent, bilirubin-binding protein, called UnaG, from the Japanese eel could be useful for a new bilirubin diagnostic assay. UnaG is an apoprotein that binds unconjugated bilirubin with high affinity and selectivity and that fluoresces upon binding. In both serum and whole blood, UnaG-based quantitation of unconjugated bilirubin was shown to be independent of conjugated bilirubin. Next steps include development of a low-cost fluorescence detector for use with the assay. <i>SciBX</i> 6(27); doi:10.1038/scibx.2013.697 Published online July 18, 2013	Patented by RIKEN; available for licensing	Kumagai, A. <i>et al. Cell</i> ; published online June 13, 2013; doi:10.1016/j.cell.2013.05.038 Contact: Atsushi Miyawaki, RIKEN Brain Science Institute, Saitama, Japan e-mail: matsushi@brain.riken.jp
Quantification of virus in T cells with latent HIV infection	A rapid method for quantifying T cells infected with latent HIV could improve the analysis of clinical samples. In blood from individuals infected with HIV, resting CD4 ⁺ T cells were purified using a bead-based affinity method and then cocultured with a CC chemokine receptor 5 (CCR5; CD195) ⁺ cell line to propagate the virus. Quantitative RT-PCR analysis of the supernatant in the coculture system revealed actively replicating HIV sooner and at a lower detection threshold than a conventional ELISA. Next steps could include using the assay in clinical trials that are evaluating therapies to eradicate latent HIV. <i>SciBX</i> 6(27); doi:10.1038/scibx.2013.698 Published online July 18, 2013	Patent and licensing status undisclosed	Laird, G.M. <i>et al. PLoS Pathog.</i> ; published online May 30, 2013; doi:10.1371/journal.ppat.1003398 Contact: Robert F. Siliciano, The Johns Hopkins University School of Medicine, Baltimore, Md. e-mail: rsiliciano@jhmi.edu
Computational models			
An algorithm for more accurate identification of cancer-associated mutations in genome sequencing studies	An improved algorithm to assess the significance of mutations identified through genome sequencing could be used to more accurately predict cancer-associated genes. A set of 3,083 matched, tumor-normal pairs was used to develop an algorithm to identify cancer-associated genes that includes correcting for heterogeneous rates of mutation across the genome. The algorithm identified cancer-associated genes with higher accuracy than an earlier iteration of the algorithm that did not correct for heterogeneous rates of mutation. Next steps include improving model accuracy using additional sequencing data (<i>see Cancer genome statistics lesson, page 7</i>). <i>SciBX</i> 6(27); doi:10.1038/scibx.2013.699 Published online July 18, 2013	Patent application filed; freely available for not-for-profit use; available for licensing for commercial use	Lawrence, M.S. <i>et al. Nature</i> ; published online June 16, 2013; doi:10.1038/nature12213 Contact: Gad Getz, Broad Institute of MIT and Harvard, Cambridge, Mass. e-mail: gadgetz@broadinstitute.org Contact: Eric S. Lander, same affiliation as above e-mail: lander@broadinstitute.org
Disease models			
Canine model for autosomal-recessive myotubular myopathy	Dogs with splicing mutations in <i>bridging integrator 1 (BIN1)</i> could be useful as models to help identify therapies for treating autosomal-recessive myotubular myopathy. Genomic analyses of three patients and five Great Danes with an inherited myopathy identified homozygous <i>BIN1</i> splicing mutations in skeletal muscle that were not observed in healthy subjects and dogs. Biopsies of muscle tissue from the affected dogs recapitulated multiple markers of disease seen in patient muscle tissue, such as nuclear internalization, fiber atrophy and membrane alterations. Next steps include establishing a population of Great Danes and smaller dog breeds harboring the <i>BIN1</i> mutation to help elucidate the pathogenesis of myotubular myopathy. <i>SciBX</i> 6(27); doi:10.1038/scibx.2013.700 Published online July 18, 2013	Unpatented; available for partnering	Böhm, J. <i>et al. PLoS Genet.</i> ; published online June 6, 2013; doi:10.1371/journal.pgen.1003430 Contact: Jocelyn Laporte, Institute of Genetics and Molecular and Cellular Biology, Illkirch, France e-mail: jocelyn@igbmc.fr

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Primary tumor xenograft mouse model for prostate cancer	A primary tumor mouse xenograft model could be useful for studying prostate cancer pathogenesis. Previous xenograft models for prostate cancer had low engraftment rates. In mice, transplantation of mouse neonatal stromal cells and primary prostate tumor cells from 12 patients with prostate cancer led to tumor engraftment in 60 of 94 animals. In these mice, tumor growth was inhibited by castration and promoted by testosterone treatment. Next steps include establishing xenograft models for other prostate cancer subtypes including castration-resistant prostate cancer. SciBX 6(27); doi:10.1038/scibx.2013.701 Published online July 18, 2013	Unpatented; licensing status not applicable	Toivanen, R. <i>et al. Sci. Transl. Med.</i> ; published online May 29, 2013; doi:10.1126/scitranslmed.3005688 Contact: Gail P. Risbridger, Monash University, Clayton, Victoria, Australia e-mail: gail.risbridger@monash.edu
Stable, patient-derived breast cancer xenograft mouse models	Patient-derived breast cancer mouse xenograft models could help identify therapeutics for triple-negative breast cancer. In immunodeficient mice, implantation of patient breast tumor fragments led to stably transplanted xenograft tissue with the same histological features as the tumor of origin. The xenografts showed stable protein and gene expression over multiple passages and had the same response to therapeutics as the source patients in 12 of 13 cases. Next steps include comparing patient and mouse responses to standard chemotherapeutics. SciBX 6(27); doi:10.1038/scibx.2013.702 Published online July 18, 2013	Unpatented; licensed to StemMed Ltd. for the exclusive use and distribution for commercial applications; available to academics from Baylor College of Medicine on a cost recovery basis	Zhang, X. <i>et al. Nature</i> ; published online June 4, 2013; doi:10.1158/0008-5472.CAN-12-4081 Contact: Michael T. Lewis, Baylor College of Medicine, Houston, Texas e-mail: mtlewis@bcm.edu
Drug delivery			
Tissue-specific drug delivery with antibody-coated, polymer, rod-shaped nanoparticles (nanorods)	Nanorods could help deliver drugs to target tissues with higher specificity than spherical nanoparticles. In cultured rat brain endothelial cells and in a microfluidic model for the vascular system lined with such cells, nanorods coated with antibody against mouse intercellular adhesion molecule-1 (Icam-1; Cd54) showed greater cellular uptake and adhesion than spherical nanoparticles coated with the same antibody. In normal mice, nanorods coated with antibodies against Icam-1 or mouse transferrin receptor showed higher accumulation in lungs and brain compared with spherical nanoparticles coated with the same respective antibodies. Next steps could include testing drug-loaded nanorods in animal models for cancer. SciBX 6(27); doi:10.1038/scibx.2013.703 Published online July 18, 2013	Patent and licensing status unavailable	Kolhar, P. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 10, 2013; doi:10.1073/pnas.1308345110 Contact: Samir Mitragotri, University of California, Santa Barbara, Calif. e-mail: samir@engr.ucsb.edu Contact: Erkki Ruoslahti, Sanford-Burnham Medical Research Institute, La Jolla, Calif. e-mail: ruoslahti@sanfordburnham.org
Drug platforms			
Crystal structure of NLR family CARD domain containing 4 (NLR4)	The crystal structure of NLR4 could help guide the rational design of compounds against the target. NLR4 is a component of the inflammasome, which helps to activate inflammatory cytokines. A crystal structure of NLR4 lacking its caspase recruitment domain (CARD) identified a region critical for maintaining the inactive, autoinhibited form of the protein and also suggested a potential ligand-induced activation mechanism. Mutagenesis studies identified specific amino acid residues that contribute to autoinhibition. Next steps include collaborating with another lab to perform a virtual screen for candidate NLR4 inhibitors. SciBX 6(27); doi:10.1038/scibx.2013.704 Published online July 18, 2013	Patent and licensing status undisclosed	Hu, Z. <i>et al. Science</i> ; published online June 13, 2013; doi:10.1126/science.1236381 Contact: Jijie Chai, Tsinghua University and the Tsinghua University-Peking University Center for Life Sciences, Beijing, China e-mail: chaijj@mail.tsinghua.edu.cn
High-yield generation of functional neurons from human induced pluripotent stem (iPS) cells	Mouse and cell culture studies suggest the transcription factor neurogenin 2 (Neurog2; Ngn2) could be used to increase the yield of neurons generated from human pluripotent stem cells. In human embryonic stem cell (hESC) and human iPS cell lines, lentiviral vector-mediated expression of Ngn2 drove the induction of neuronal cells with near 100% yield and purity in less than 2 weeks. In culture, these cells formed synapses, and when cocultured with mouse cortical neurons, the cells integrated into synaptic networks. In newborn mice, transplanted cells functionally integrated into the mouse brain at six weeks. Next steps could include evaluating the method for the study of disease-specific mutations and drug vulnerabilities. SciBX 6(27); doi:10.1038/scibx.2013.705 Published online July 18, 2013	Patent and licensing information unavailable	Zhang, Y. <i>et al. Neuron</i> ; published online June 5, 2013; doi:10.1016/j.neuron.2013.05.029 Contact: Thomas C. Südhof, Stanford University School of Medicine, Stanford, Calif. e-mail: tcs1@stanford.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
IL-12-expressing oncolytic herpes virus to treat glioblastoma	<p>Mouse studies identified an oncolytic virus expressing IL-12 that could help treat glioblastoma. In a mouse model for glioma stem cell-induced glioblastoma, an oncolytic herpes virus expressing a mouse <i>Il-12</i> transgene inhibited tumor growth and increased survival compared with a virus expressing no transgene or saline. In this model, the virus expressing IL-12 also inhibited angiogenesis and induced a T cell-mediated immune response against the cancer cells. Next steps include creating an oncolytic virus that could be evaluated in clinical trials.</p> <p>SciBX 6(27); doi:10.1038/scibx.2013.706 Published online July 18, 2013</p>	Patented by Georgetown University and Massachusetts General Hospital; licensed to Catherex Inc.; Massachusetts General Hospital patents available for licensing	<p>Cheema, T.A. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online June 10, 2013; doi:10.1073/pnas.1307935110 Contact: Samuel D. Rabkin, Massachusetts General Hospital and Harvard Medical School, Boston, Mass. e-mail: rabkin@helix.mgh.harvard.edu</p>
Stabilization of enzymes with polycationic branched polymers	<p>Stabilization of enzymes with polycationic branched polymers could improve the oral delivery of therapeutics. Poly-(3,5-bis(3-aminopropoxy)benzyl)-methacrylate was conjugated to a bacteria-derived, proline-specific endopeptidase. In rats, the endopeptidase-polymer conjugate showed greater retention and catalytic activity within the stomach than the same enzyme conjugated to polymers that stabilize proteins for systemic delivery. Next steps include evaluating the impact of polymer structure, molecular weight and conjugation site on efficacy and safety.</p> <p>SciBX 6(27); doi:10.1038/scibx.2013.707 Published online July 18, 2013</p>	Unpatented; licensing status not applicable	<p>Fuhrmann, G. <i>et al. Nat. Chem.</i>; published online June 9, 2013; doi:10.1038/nchem.1675 Contact: Jean-Christophe Leroux, Swiss Federal Institute of Technology Zurich (ETHZ), Zurich, Switzerland e-mail: jlroux@ethz.ch</p>
Universal, chimeric antigen receptor (CAR)-based T cell therapy using zinc finger nuclease (ZFN) T cell editing	<p>T cells engineered to express a CD19-specific CAR and lack major histocompatibility complex class I A (HLA-A) could be used as a universal therapy to treat CD19⁺ cancers. Allogeneic, CAR-based T cell therapies are limited because of potential rejection of the engineered donor cells by the recipient. In human, CD19-specific CAR T cells, ZFN mRNAs were introduced by electrotransfer and shown to eliminate HLA-A expression during 50 days of coculture. The modified CD19-specific T cells evaded HLA-A-restricted cytotoxic T lymphocyte attack and maintained antitumor activity against patient-derived primary lymphoma cells. Next steps include clinical trials using CD19-specific T cells that lack T cell receptor (TCR) expression and CD19-specific T cells that lack TCR and HLA-A expression.</p> <p>SciBX 6(27); doi:10.1038/scibx.2013.708 Published online July 18, 2013</p>	Patent application filed; available for licensing from The University of Texas MD Anderson Cancer Center Office of Technology Commercialization Contact: Emmanuelle Schuler, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: eschuler@mdanderson.org	<p>Torikai, H. <i>et al. Blood</i>; published online June 5, 2013; doi:10.1182/blood-2013-03-478255 Contact: Laurence J.N. Cooper, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: ljncooper@mdanderson.org</p>

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