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# Dissociating the effects of A<sub>2A</sub> agonism

By Kai-Jye Lou, Staff Writer

The vasodilatory effects of adenosine A<sub>2A</sub> receptor agonists make them useful as cardiac stress agents in imaging but have made it difficult to exploit these molecules' potent anti-inflammatory effects.<sup>1</sup> Researchers in Germany have dissociated the anti-inflammatory effects of adenosine A<sub>2A</sub> receptor agonists from their hemodynamic effects and are determining a specific inflammation-related indication to pursue with the molecule.<sup>2</sup>

Activation of the adenosine A<sub>2A</sub> receptor (ADORA<sub>2A</sub>) on endothelial cells promotes vasodilation, and activation of the receptor on immune cells inhibits inflammatory processes. The vasodilatory effects become dose-limiting side effects in the inflammation setting by causing hypotension, which in turn triggers a compensatory increase in heart rate and cardiac output.

Thus, Jürgen Schrader and colleagues at the **Heinrich Heine University of Duesseldorf** and the **University of Bonn** have been looking at the endogenous adenosine signaling pathway for molecular targets that can be exploited to dissociate the vasodilatory effects of ADORA<sub>2A</sub> agonists from their anti-inflammatory effects.

The search led the group to ecto-5'-nucleotidase (NT5E; NT; CD73), which dephosphorylates adenosine monophosphate (AMP) to form adenosine<sup>3,4</sup> (see **Figure 1**, "Effects of adenosine A<sub>2A</sub> receptor agonism").

Schrader, a professor of physiology and head of the Department of Molecular Cardiology at the Heinrich Heine University of Duesseldorf, said the group was drawn to CD73 because of the enzyme's expression under normal and inflammatory conditions.

"We've previously observed that whenever there is inflammation, immune cells dramatically increase their expression of both CD73 and the A<sub>2A</sub> receptor," he told *SciBX*. "So our idea is why not make a phosphorylated A<sub>2A</sub> receptor agonist prodrug that remains biologically inactive until it is locally activated by CD73 at sites of inflammation."

In 2009, the researchers published data on a series of AMP derivatives and showed *in vitro* that the molecules were accepted as substrates by mouse Cd73 and dephosphorylated into the corresponding ADORA<sub>2A</sub> agonist.<sup>5</sup>

**"If we could show in these other disease models that our A<sub>2A</sub> receptor prodrug works equally well as known A<sub>2A</sub> receptor agonists but without the hemodynamic side effects, it could considerably broaden its application in inflammation."**

—Jürgen Schrader,  
Heinrich Heine  
University of Duesseldorf

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Now, the group has shown *in vivo* that one of the lead derivatives from the 2009 study, 2-cyclohexylethylthio-AMP (chet-AMP), is selectively activated at sites of inflammation and has negligible vasodilatory effects.

In mouse models of collagen-induced arthritis, delivery of chet-AMP via implanted osmotic minipumps decreased joint inflammation compared with no treatment. Importantly, plasma chet-AMP concentrations that had an anti-inflammatory effect were in the subnanomolar range and about 100-fold lower than the concentrations required for vasodilation.

Results were published in *Science Translational Medicine*.

**Inflammatory history lessons**

The prodrug strategy could help overcome a key barrier that has caused some ADORA<sub>2A</sub> agonists to flounder in the inflammation setting.

“The hemodynamic effects of A<sub>2A</sub> receptor agonists limit their dosing to the point where they are no longer effective at resolving inflammation, and this has contributed to the failure of several such compounds,” said Schrader.

Examples of discontinued ADORA<sub>2A</sub> agonists include GW328267X from **GlaxoSmithKline plc** and UK-432097 from **Pfizer Inc.** Both compounds were being developed to treat chronic obstructive pulmonary disorder (COPD) but were discontinued in Phase II trials due to poor efficacy.

At least two ADORA<sub>2A</sub> agonists are still in clinical trials for inflammation-related indications. BVT.115959, which is being developed by **CBT Development Ltd.**, **Ergomed Group** and **Swedish Orphan Biovitrum AB**, is in Phase II testing to treat diabetic neuropathic pain. Lexiscan regadenoson is being evaluated in an investigator-led Phase I trial to treat sickle cell disease-associated pain crisis.

Holger Eltzschig, a professor of anesthesiology, medicine, cell biology and immunology at the **University of Colorado Denver School of Medicine**, said targeting ADORA<sub>2A</sub> with a specific agonist could have an advantage over drugs that work via adenosine liberation, such as methotrexate. The specific compounds, he said, could be more effective at activating the receptor and may not be affected by pathways that rapidly shut down adenosine signaling.

**Gilead Sciences Inc.** and **Astellas Pharma Inc.** already market Lexiscan as a cardiac stress agent for use in myocardial perfusion imaging (MPI).

Despite the multitude of ADORA<sub>2A</sub> agonists, Schrader cautioned that the group’s prodrug strategy cannot be generalized to any ADORA<sub>2A</sub> agonist. “The phosphorylated prodrug needs to be compatible as a substrate for CD73,” he told *SciBX*.

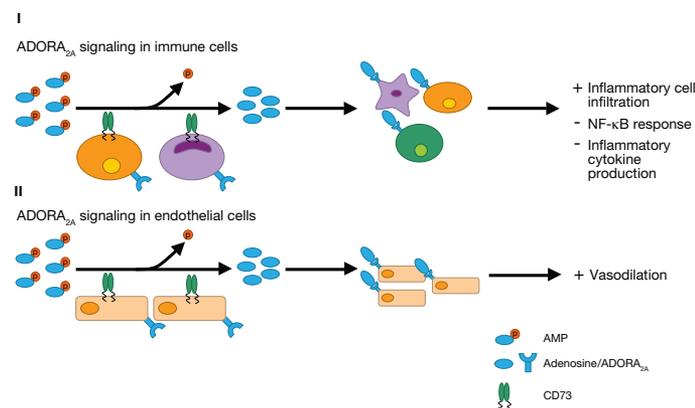
**For your inflammation**

Schrader said the group’s key step forward will be to select an appropriate inflammation-related indication to pursue with chet-AMP.

Bruce Cronstein, a professor in the Department of Medicine and Pathology and the Department of Biochemistry and Molecular Pharmacology at the **New York University Langone Medical Center**, thinks the ADORA<sub>2A</sub> agonist prodrug could be applied in chronic inflammation settings because it may be selectively activated at sites of inflammation.

Cronstein suggested that the researchers develop their compound in indications for which marketed anti-inflammatory drugs are not particularly effective. “Where they may want to head is to conditions such as psoriatic arthritis and ankylosing spondylitis,” he told *SciBX*.

Joel Linden, professor of inflammation biology at the **La Jolla Institute for Allergy & Immunology**, liked the idea that chet-AMP could be used



**Figure 1. Effects of adenosine A<sub>2A</sub> receptor agonism.** Activation of the adenosine A<sub>2A</sub> receptor (ADORA<sub>2A</sub>) is known to both inhibit inflammatory processes and promote vasodilation. The cell surface enzyme ecto-5'-nucleotidase (NT5E; NT; CD73) is highly expressed by immune cells and endothelial cells and is one of the central regulators of endogenous adenosine signaling. CD73 dephosphorylates adenosine monophosphate (AMP) to form adenosine.

(I) In immune cells, adenosine signaling through ADORA<sub>2A</sub> inhibits proinflammatory processes.

(II) In endothelial cells, adenosine signaling through ADORA<sub>2A</sub> promotes vasodilation.

In Flögel *et al.*, researchers showed that their ADORA<sub>2A</sub> agonist prodrug is selectively activated at sites of inflammation, at which CD73 is highly upregulated. Thus, the molecule is able to exert potent anti-inflammatory effects at doses that do not also cause vasodilation.

to create high local concentrations of an active ADORA<sub>2A</sub> agonist at sites of inflammation and thinks it is a clever and appealing approach to limit the exposure of other tissues to the active form of the compound.

However, he noted that because the prodrug compound is phosphorylated, it would most likely need to be delivered intravenously and in a hospital setting, which could make it difficult to develop for chronic inflammatory conditions.

Linden therefore thinks chet-AMP would be best suited for acute inflammation, such as treating acute flare-ups in patients with rheumatoid arthritis (RA).

Linden wanted to see additional examples of ADORA<sub>2A</sub> agonists that incorporate the CD73-activated prodrug strategy described by the

researchers. He added that it also will be a good idea to determine whether chet-AMP could be formulated for oral delivery, or as a subcutaneous injection or implant.

Cronstein added that it will be important to show that chet-AMP is a substrate for human CD73 and to demonstrate that there would be no acute effects on blood pressure and bleeding in humans.

Finally, Eltzschig wanted to know whether the prodrug strategy could be applied to an ADORA<sub>2B</sub> agonist, as such compounds also are known to have potent anti-inflammatory effects.

Schrader said the group now is looking at CD73 and ADORA<sub>2A</sub> expression patterns in mouse models of other inflammation-related conditions, such as ischemia/reperfusion injury and sepsis, to help determine whether chet-AMP could be useful in such settings.

"If we could show in these other disease models that our A<sub>2A</sub> receptor prodrug works equally well as known A<sub>2A</sub> receptor agonists but without the hemodynamic side effects, it could considerably broaden its application in inflammation," he told *SciBX*.

The Heinrich Heine University of Duesseldorf and the University of Bonn have cofiled a patent covering phosphorylated ADORA<sub>2A</sub> agonists. The work is available for licensing.

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## REFERENCES

- Haskó, G. *et al. Nat. Rev. Drug Discov.* **7**, 759–770 (2008)
- Flögel, U. *et al. Sci. Transl. Med.*; published online Aug. 8, 2012; doi:10.1126/scitranslmed.3003717
- Deaglio, S. *et al. J. Exp. Med.* **204**, 1257–1265 (2007)
- Kobie, J.J. *et al. J. Immunol.* **177**, 6780–6786 (2006)
- El-Tayeb, A. *et al. J. Med. Chem.* **52**, 7669–7677 (2009)

## COMPANIES AND INSTITUTIONS MENTIONED

**Astellas Pharma Inc.** (Tokyo:4503), Tokyo, Japan  
**CBT Development Ltd.**, Cambridge, U.K.  
**Ergomed Group**, Frankfurt, Germany  
**Gilead Sciences Inc.** (NASDAQ:GILD), Foster City, Calif.  
**GlaxoSmithKline plc** (LSE:GSK; NYSE:GSK), London, U.K.  
**Heinrich Heine University of Duesseldorf**, Duesseldorf, Germany  
**La Jolla Institute for Allergy & Immunology**, La Jolla, Calif.  
**New York University Langone Medical Center**, New York, N.Y.  
**Pfizer Inc.** (NYSE:PFE), New York, N.Y.  
**Swedish Orphan Biovitrum AB** (SSE:SOBI), Stockholm, Sweden  
**University of Bonn**, Bonn, Germany  
**University of Colorado Denver School of Medicine**, Denver, Colo.

# The cost of reproducibility

By *Tim Fulmer, Senior Writer*

It is no secret that industry scientists often cannot reproduce research published by others, but approaches to solving the problem have been scarce and usually involve a would-be investor footing the bill for additional studies. The newly launched Reproducibility Initiative has a different approach and looks to put the onus on the originating academics to pay for confirmatory experiments. Precise details regarding funding, standards of reproducibility and potential conflicts of interest have yet to be worked out.

Earlier this month, research service provider **Science Exchange** partnered with publisher **PLOS** and the open-access data repository **figshare** to launch the initiative, which lets researchers apply to Science Exchange to have their studies replicated by a CRO or a university lab facility. When the replication study is completed, the researchers have the option of uploading the new results to figshare and publishing them in the online journal *PLoS ONE*, which will contain a link to the publication in which the original experiments appeared.

figshare is part of the digital science division of Macmillan Publishing, which is the parent company of Nature Publishing Group.

Researchers also will receive a certificate indicating their results were successfully replicated.

The researchers are responsible for all costs associated with the validation study, in addition to a 5% transaction fee paid to Science Exchange for handling communication, data transfer and payment between the researchers and the CRO.

Whether funding agencies would welcome such a mechanism or would rather up the ante on the quality of the initial research remains to be seen.

“Ultimately, we would like to see funding agencies step in and help fund this initiative,” Science Exchange cofounder and CEO Elizabeth Iorns told *SciBX*. “But in the meantime, we think we have incentives in place to encourage researchers to fund the validation work themselves. First of all, the researchers get an additional publication in *PLoS ONE*. Secondly, they receive a certificate that provides proof of third-party validation to potential licensing partners and investors,” she said.

“Finally, we estimate the validation study could cost as low as 10% of the total cost of a lab’s research budget for the findings in a particular paper. That’s because the goal is not to replicate all of the preliminary and exploratory work that is part of most research programs. Rather, the goal is to replicate only those few key published experiments that underlie the translational potential of a particular set of findings,” concluded Iorns.

The scientific advisory board (SAB) of Science Exchange will calculate the total cost for a validation study based on which experiments require replication and which of the more than 1,000 CROs and university lab facilities in the Science Exchange network is best equipped and qualified to carry out the study.

The 10 individuals on the SAB are Iorns; Bruce Booth, partner at **Atlas Venture**; Lee Ellis, professor of surgery at **The University of Texas MD Anderson Cancer Center**; John Ioannidis, professor of medicine at

the **Stanford University School of Medicine**; Bernard Munos, founder of the **InnoThink Center for Research in Biomedical Innovation**; Brian Nosek, professor of psychology at the **University of Virginia**; Heather Piwowar, postdoctoral researcher at **Duke University**; George Robertson, professor of psychiatry at **Dalhousie University**; G. Sitta Sittampalam, professor of pharmacology and toxicology at **The University of Kansas Medical Center**; and Victoria Stodden, professor of statistics at **Columbia University**.

If the original researchers agree to the cost proposed by the SAB, they are then allowed to communicate with the CRO only through Science Exchange, which acts as an intermediary throughout the process. That arrangement ensures that the researchers cannot influence the outcome of the confirmatory study.

Once the validation study is completed, the results are presented to the original researchers and to the SAB. Finally, the SAB determines whether or not the original experiments were successfully replicated.

If the experiments are not replicated, the researchers can appeal to have a second CRO attempt to replicate them—again at the researchers’ cost. A second failure to replicate is considered strong proof that the original experiments are not reproducible. At that point, it is entirely up to the original researchers whether to disclose the failure to reproduce and/or to retract the original publication. The initiative has no authority to demand a retraction based on a failure to replicate.

Iorns did not comment on whether there are any concerns that conflicts of interest and ethical issues may arise as a result of SAB members being privy to confidential information on papers that do not pass the reproducibility test and for which the authors decide not to publish negative results.

The entire validation study is carried out under a confidentiality agreement, and the researchers are not obligated to make the new data public. However, Science Exchange encourages them to publish the results in the *PLoS ONE* Reproducibility Collection and to post them on figshare.

The purpose of the collection is to provide a venue for the publication of studies that reproduce published work, either confirming or refuting the original result, said *PLoS ONE* associate editor Elizabeth Silva.

“We are one of only a handful of publications that actively encourage these types of submissions; traditionally they have been extremely difficult to get published, mainly because journals don’t feel they are novel or exciting enough,” she said.

The Reproducibility Initiative will accept 40–50 studies initially for validation. The SAB “is looking for studies with clear translational potential that have, for example, identified a putative cancer target. Ideally, the studies will have used standard models and methods that should be straightforward to replicate in a CRO, such as mouse xenografts or cell culture lines and methods such as an ELISA, mass spec or genomic analysis,” said Iorns.

Science Exchange plans to publish a meta-analysis of all the studies that looks for trends of reproducibility and non-reproducibility across

**“Ideally, the studies will have used standard models and methods that should be straightforward to replicate in a CRO, such as mouse xenografts or cell culture lines and methods such as an ELISA, mass spec or genomic analysis.”**

**—Elizabeth Iorns,  
Science Exchange**

various experiments, said Iorns. Researchers can choose to have their names withheld from that analysis.

Deciding whether or not a paper has been successfully replicated could turn into a more complex discussion if the results of the reproducibility study are mixed—how the SAB will handle such situations remains to be determined.

Data obtained from those first studies “will give us a sense in real life how ‘non-reproducible’ manifests itself and in how many different versions and degrees of severity,” said Ioannidis. “Any discrepancies between the initial experiments and the reproducibility ones will be carefully scrutinized to find out what happened and why.”

#### The venture side

The Reproducibility Initiative could help entrepreneurs and early stage investors by saving time and money that can be used to move the technology forward rather than to do third-party validation, Daphne Zohar told *SciBX*.

“A technology that already has third-party validation data would be more attractive to investors than one that doesn’t,” she said.

Zohar is founder and managing partner of **PureTech Ventures**, a VC that specializes in funding early stage life sciences research. PureTech typically has key experiments repeated by third parties, using CROs and sometimes academic labs, said Zohar.

In March, a group at **Amgen Inc.** claimed the findings of only 6 of 53 (11%) papers deemed landmark studies by the hematology and oncology research communities were able to be confirmed by industry scientists.<sup>1</sup> Similarly, in 2011, a team from **Bayer AG**’s Bayer HealthCare

subsidiary reported that only 25% of published preclinical studies could be validated.<sup>2</sup>

In 2011, Booth told *SciBX* that the “unspoken rule” among early stage VCs is that at least 50% of published studies cannot be repeated by an industrial lab. As a result, Atlas insists on external validation studies of a new company’s basic science as a precondition to further investment.<sup>3</sup>

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#### REFERENCES

1. Begley, C.G. & Ellis, L.M. *Nature* **483**, 531–533 (2012)
2. Prinz, F. *et al. Nat. Rev. Drug Discov.* **10**, 712 (2011)
3. Osherovich, L. *SciBX* **4**(15); doi:10.1038/scibx.2011.416

#### COMPANIES AND INSTITUTIONS MENTIONED

**Amgen Inc.** (NASDAQ:AMGN), Thousand Oaks, Calif.

**Atlas Venture**, Boston, Mass.

**Bayer AG** (Xetra:BAYN), Leverkusen, Germany

**Columbia University**, New York, N.Y.

**Dalhousie University**, Halifax, Nova Scotia, Canada

**Duke University**, Durham, N.C.

**figshare**, London, U.K.

**InnoThink Center for Research in Biomedical Innovation**, Indianapolis, Ind.

**PLOS**, San Francisco, Calif.

**PureTech Ventures**, Boston, Mass.

**Science Exchange**, Palo Alto, Calif.

**Stanford University School of Medicine**, Palo Alto, Calif.

**The University of Kansas Medical Center**, Kansas City, Kan.

**University of Virginia**, Charlottesville, Va.

**The University of Texas MD Anderson Cancer Center**, Houston, Texas



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# deCode-ing autism

By Lev Osherovich, Senior Writer

Recent general media coverage has suggested a gene sequencing study by **deCode genetics ehf** showed a clear link between paternal age and mutations that might cause autism spectrum disorder and schizophrenia. However, the newspapers failed to note that a direct relationship between the mutations described in the paper and autism spectrum disorder or schizophrenia remains to be established. The papers also glossed over the main finding of the study—there is a paternal origin for genetic variation in the general human population.

The deCode-led team used high throughput gene sequencing to identify new mutations in the genomes of a handful of individuals that are absent in their parents and thus are likely to have spontaneously arisen in the father's sperm.<sup>1</sup> Their analysis revealed an intriguing correlation between number of mutations and father's age.

The general media described the findings as a breakthrough in understanding the roots of autism spectrum disorder (ASD) and schizophrenia but missed the true value of the study as a snapshot of human evolution in action.

"This paper sheds much less light on the pathogenesis of schizophrenia and autism than the lay press has indicated," said the study's coauthor, deCode CEO Kári Stefánsson. "This is mainly a study of nucleotide mutations in human populations."

The study quantifies the appearance of new mutations from one generation to the next and suggests that these mutations could contribute to ASD and schizophrenia, two neuropsychiatric disorders thought to have a strong genetic underpinning.

However, much more work is needed to prove that the mutations identified by the deCode team really cause neuropsychiatric disease in a broader patient population.

Answering how much these mutations really contribute to ASD and schizophrenia risk will require analysis of a much larger cohort of patients and their families.

## Shown and known

Stefánsson's team gathered complete genomic data from 78 Icelandic families typically consisting of a father, a mother and their offspring. Of the offspring, 44 were autistic and 21 had schizophrenia. Altogether the team sequenced 219 individuals.

The team found a total of 4,933 SNPs in the offspring that were absent in both parents and were thus likely to have arisen at or around conception or early embryonic development. The average individual had 63.2 such *de novo* or spontaneous mutations that differed from the parents' genomes.

Notably, the children of older fathers had the highest number of *de novo* mutations. Each year of the father's age correlated with a cumulative increase of about two mutations. Advanced maternal age did not correlate with a higher mutation count.

Stefánsson thus proposed that mutation in the sperm of older fathers accounts for the majority of new genetic variation.

"This is the first paper describing genome studies of mutations that increase with age," said Stefánsson. "If you ask what causes mutation in a population, 97% of it is explained by the age of the father."

Results were published in *Nature* and are not patented.

## Disease connection?

The deCode study builds a solid case for a paternal origin of spontaneous genetic variation, thought to be a basic mechanism of evolution. Whether these paternally derived mutations affect ASD and schizophrenia risk remains unclear.

The idea that mutations in general contribute to ASD and schizophrenia is not new. One leading theory argues that the two conditions are caused by individually rare but collectively numerous genetic variants. These variants alter the activity of proteins involved in neurological development, leading to problems in brain connectivity and cognitive functioning.

Consistent with this genetic theory, recent studies by multiple academic teams have turned up a host of chromosomal abnormalities that are likely to underlie certain highly heritable forms of ASD.<sup>2</sup>

However, not all cases of ASD can be accounted for by such gross genetic alterations, prompting a search for more subtle genetic variants such as disease-associated SNPs and copy number variants.

Mark Daly, associate professor of medicine at **Harvard Medical School** and an associate member of the **Broad Institute of MIT and Harvard**, said ASD and schizophrenia have a very high level of heritability, "more than 50%, perhaps as high as 80%," and thus are likely to have a strong genetic basis.

However, the deCode study focuses on new spontaneous genetic variants that are not present in the parents and thus would have been overlooked in prior studies of heritable factors in ASD.

Daly suspects that many cases of ASD are caused by a combination of inherited and spontaneous mutations, but unraveling the relative contribution of these two types of mutations is technically challenging.

Earlier this year, teams led by Daly and others sequenced the exomes of patients with ASD and uncovered rare *de novo* mutations in known ASD-linked genes and new ASD candidate genes.<sup>3-5</sup>

Because numerous prior epidemiological studies have established that paternal age is a major risk factor for ASD, "it is beyond reasonable doubt that the risk of autism increases with the age of the father," said Stefánsson. "What is controversial is how large a percentage of this increase is explained" by *de novo* mutations such as those uncovered in the deCode study.

Stefánsson proposed that new mutations in the sperm of older fathers could be a risk factor for neuropsychiatric disease. Based on a statistical model, his team proposed that the increased mutational rate caused by high paternal age accounted for the high ASD risk that prior epidemiological studies had linked to delayed parenthood.

Because both the age at reproduction and rates of ASD have steadily crept up in industrialized nations, he speculated that increased mutation rates could thus contribute to the rise in ASD.

"The elusive question is what has increased the rate of autism diagnosis," said Stefánsson. "I think the age of the father explains some of it but not all of it."

Daly said it is hard to extrapolate the overall impact of paternal age-related *de novo* mutations on ASD risk.

"There's a lot of epidemiology on the effect of increasing age of parents on autism risk, but different studies estimate that effect at different strengths," he said.

"We know some cases of ASD arise from spontaneous point mutations. This paper shows the rate of such mutations increases with paternal age," said Daly. "While some of these mutations will without question contribute

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# Eliminating teratomas

By Lauren Martz, Staff Writer

A **University of California, San Diego** team has developed a genetic method to prevent embryonic stem cell therapies from forming cancerous teratomas following transplantation.<sup>1</sup> The method was safe in mice, although questions remain about whether the genetic modifications will raise safety and regulatory concerns in patients.

Teratomas are a type of tumor tissue that result from the abnormal development of pluripotent cells when transplanted *in vivo*. They are usually benign, but malignant teratomas can occur.

In human embryonic stem cell (hESC)-based therapies, pluripotent stem cells are differentiated into cells of a desired type that are then transplanted into patients. Some of the cells may not fully differentiate, which results in cell cultures containing some fraction of undifferentiated pluripotent cells that could form malignant teratomas upon transplantation.

The challenge is ensuring that a stem cell-based therapy contains only differentiated cells prior to transplantation.

Two common approaches to the problem have been cell sorting, which

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(Continued from “deCode-ing autism,” p. 6)

to ASD risk, what’s not established is whether this increase in risk is tiny or substantial.”

Daniel Geschwind, professor of neurology and psychiatry and chair of human genetics at the **University of California, Los Angeles**, said the sample size in Stefánsson’s study was too small to draw firm conclusions about the impact of *de novo* mutations on ASD and schizophrenia risk.

“The fact the paternal age can account for the majority of mutation in offspring makes this paper *Nature*-worthy,” said Geschwind. “But replicating this with a larger data set prior to publication would have been nice.”

Demonstrating that paternal age leads to specific mutations in known ASD genes would help Stefánsson’s case. Geschwind suggested the deCode team “narrow down the relationship between these mutations and actual autism” by identifying and characterizing individual mutations that are likely to cause ASD in the sequenced individuals.

## Alternative theories

There are multiple other possibilities for why neuropsychiatric disease rises in the children of older parents.

Daly cautioned that it is unclear whether the rise in ASD diagnoses over the last few decades is due to biological mechanisms such as higher mutation rates or from social factors.

Widening of diagnostic criteria and greater awareness of the disorder may in fact contribute to the prevalence of ASD, which the **Centers for Disease Control and Prevention** recently estimated might affect 1 in 88 children.

“There is now a broader definition of autism that allows diagnosis in a larger number of people,” said Stefánsson.

“There is lack of agreement in the field about the rate at which ASD is increasing over and above the shift in diagnosis,” added Daly.

removes any pluripotent cells prior to transplantation, or antibody-based cytotoxic therapies that target pluripotent cells. However, cell sorting can damage the differentiated cells, and antibody-based targeting requires identification of pluripotent cell-specific surface antigens.

Now, a UCSD team led by Yang Xu, a professor of molecular biology, has developed a genetic approach to prevent teratoma formation. The approach is based on prior genetic work by other labs that used viral vectors to express an inducible suicide gene in pluripotent cells, thereby eliminating the cells and reducing the risk of teratoma formation.

In that prior work, groups at **The Hebrew University of Jerusalem** and **Sun Yat-Sen University** induced expression of herpes simplex virus thymidine kinase (HSV-tk) in hESCs using different genetic methods.<sup>2,3</sup> The alteration rendered the cells sensitive to **Roche’s** Cytovene ganciclovir, a drug for cytomegalovirus that targets HSV-tk.

Although those two methods prevented teratoma formation in mice, both used viral-based gene delivery, which itself creates the risk of random genetic insertions and mutagenesis that can lead to cancer. In addition, ganciclovir was toxic to healthy cells at the high concentrations used.

The UCSD team hypothesized that one way to avoid those problems might be to use a mutant form of the *HSV-tk* gene that rendered cells hypersensitive to ganciclovir and then insert that gene into a locus in the genome of the pluripotent cells using homologous recombination.

(Continues on p. 8)

Also, greater public awareness of the disease may lead some parents to suspect an autism diagnosis, and older parents are more likely to seek medical treatment for children.<sup>6</sup>

Another possibility is that male carriers of mild hereditary forms of the disease may delay reproduction due to social difficulties in finding mates.

“Older fathers may be on the autism spectrum themselves,” said Geschwind.

Stefánsson said he hopes to collaborate with Daly and other ASD researchers to test larger patient cohorts for evidence of paternally derived disease-linked mutations.

He noted that the findings are unrelated to deCode’s principal commercial efforts in discovery and development of diagnostic biomarkers.

Oshrovich, L. *SciBX* 5(34); doi:10.1038/scibx.2012.889

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## REFERENCES

1. Kong, A. *et al. Nature*; published online Aug. 22, 2012; doi:10.1038/nature11396  
**Contact:** Kári Stefánsson, deCode genetics ehf, Reykjavik, Iceland e-mail: [kstefans@decode.is](mailto:kstefans@decode.is)
2. Malhotra, D. & Sebat, J. *Cell* **148**, 1223–1241 (2012)
3. Neale, B.M. *et al. Nature* **485**, 242–245 (2012)
4. Sanders, S.J. *et al. Nature* **485**, 237–241 (2012)
5. O’Roak, B.J. *et al. Nature* **485**, 246–250 (2012)
6. Van Meter, K.C. *et al. Autism Res.* **3**, 19–29 (2010)

## COMPANIES AND INSTITUTIONS MENTIONED

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**Centers for Disease Control and Prevention**, Atlanta, Ga.  
**deCode genetics ehf**, Reykjavik, Iceland  
**Harvard Medical School**, Boston, Mass.  
**University of California, Los Angeles**, Calif.

In various hESC lines, the team inserted the mutant *HSV-tk* gene into the 3' untranslated region of the *Nanog homeobox (NANOG)* gene, which is specifically and highly expressed in pluripotent and partially differentiated cells but not in fully differentiated cells. The group used bacterial artificial chromosome (BAC)-based targeting for homologous recombination.

In culture with ganciclovir, mutant *HSV-tk*-expressing hESCs (TK-hESCs) were eliminated, whereas unmodified parental hESCs were unaffected. Without ganciclovir, TK-hESCs proliferated and differentiated as normal, suggesting the drug caused inducible hESC-specific cell death.

In immunocompromised mice, transplanted TK-hESCs or parental hESCs formed teratomas. Ganciclovir treatment beginning one day after hESC transplantation prevented teratoma formation from TK-hESCs but not from parental cells.

To more closely mimic clinical conditions, the team next transplanted partially differentiated TK-hESCs into mice and showed that ganciclovir prevented teratomas from cells derived from TK-hESCs but not from parental hESCs.

Finally, the group carried out spiking studies to determine whether treatment with ganciclovir could eliminate very small numbers of undifferentiated TK-hESCs in culture. In cultured fibroblasts, ganciclovir eliminated even a single TK-hESC.

“This work is the first step to prove that the strategy works. Next, they need to do more than prove that it can eliminate teratomas. They need to prove that it can allow differentiation of hESCs into desired therapeutic cell types that have preserved viability and function

after differentiation without causing side effects to those cells,” said Weidong Le, professor of neurology at **Baylor College of Medicine**.

“In order to prove this, they would have to test the hESC-derived cells in particular animal models of disease such as transplantation of differentiated neurons into the CNS in models of neurodegenerative disorders,” continued Le. “For neurological diseases, they would also need to prove that the neurons can populate the brain tissue, that the drug they use can penetrate the brain and target the desired gene and that it can prevent the formation of teratomas.”

### Regulatory hurdles

Xu said his team plans to determine whether the strategy can eliminate the risk of teratomas during hESC-based therapy for human diseases such as type 1 diabetes.

In addition to confirming the efficacy of the hESC-derived cells in disease models, the team will need to perform extensive safety studies.

“This strategy uses foreign DNA and genetically modified cells, which will induce scrutiny from the regulatory agencies and will be hard to get into the clinic,” said Robert Lanza, CSO of **Advanced Cell Technology Inc.**

“Although this strategy improves safety by eliminating the undifferentiated cells to possibly reduce teratoma risk, it may introduce other dangers such as increasing mutagenesis.”

Xu countered that the use of gene targeting by homologous recombination should reduce or eliminate the risks associated with other strategies to genetically modify the cells, such as virus-mediated gene delivery.

“Unlike the cancer risk associated with viral vectors that are integrated into the genome randomly, the *TK* gene is introduced into one specific locus,” he said. “We will perform whole-genome sequencing to ensure that no other mutations are introduced in knock-in hESCs. Once it is confirmed that no other random integration occurs in the knock-in hESCs via whole-genome sequencing, those genetically modified hESCs will be suitable for human use.”

Advanced Cell Technology has hESC-derived retinal pigment epithelium (RPE) cells in Phase I testing to treat Stargardt’s disease and dry age-related macular degeneration (AMD). Lanza noted that RPE cells have a rigorous differentiation process that allows the formation of a fully and terminally differentiated RPE cell population from hESCs in culture. The company also has developed an assay to detect undifferentiated hESCs in culture prior to clinical use.

Lanza told *SciBX* that the newly published technology will likely require additional purification steps prior to use in humans. “What the agencies will require is a pure homogeneous population of cells,” he said. “If you have an important cell type that is not able to be fully differentiated in culture, a strategy like this could be useful in eliminating the undifferentiated population. The problem still remains that there could be cell types other than undifferentiated cells that contaminate the culture. hESCs are capable of differentiating into any cell type, so while the proposed approach can eliminate undifferentiated cells, it does not ensure that a cell population is homogeneous.”

According to Xu, his team’s “approach addresses the issue of teratoma risk but does not help the purity of the lineage-specific differentiation. To obtain homogenous cells, the differentiated cells can be purified with cell type-specific antibodies or with knock-in hESCs with drug-resistant genes introduced into the lineage-specific locus.”

Xu told *SciBX* that the IP has not been patented and is available for licensing.

**Martz, L. *SciBX* 5(34); doi:10.138/scibx.2012.890  
Published online Aug. 30, 2012**

### REFERENCES

1. Rong, Z. *et al. J. Biol. Chem.*; published online Aug. 4, 2012; doi:10.1074/jbc.M112.383810  
**Contact:** Yang Xu, University of California, San Diego, La Jolla, Calif.  
e-mail: [yangxu@ucsd.edu](mailto:yangxu@ucsd.edu)
2. Schuldiner, M. *et al. Stem Cells* **21**, 257–265 (2003)
3. Cheng, F. *et al. Biomaterials* **33**, 3195–3204 (2012)

### COMPANIES AND INSTITUTIONS MENTIONED

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**Baylor College of Medicine**, Houston, Texas  
**The Hebrew University of Jerusalem**, Jerusalem, Israel  
**Roche** (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland  
**Sun Yat-Sen University**, Guangzhou, China  
**University of California, San Diego**, La Jolla, Calif.

**“If you have an important cell type that is not able to be fully differentiated in culture, a strategy like this could be useful in eliminating the undifferentiated population. The problem still remains that there could be cell types other than undifferentiated cells that contaminate the culture.”**

**—Robert Lanza,  
Advanced Cell Technology Inc.**

## This week in therapeutics

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

This week in therapeutics includes important research findings on targets and compounds, grouped first by disease class and then alphabetically by indication.

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
<b>Cancer</b>				
Acute lymphoblastic leukemia (ALL)	Notch 1 (NOTCH1); microRNA-181a (miR-181a); miR-181b-1	<p>Mouse and cell culture studies suggest inhibiting miR-181a and miR-181b-1 could help treat NOTCH1 pathway-driven T cell ALL. In mouse models of the disease, miR-181a and miR-181b-1 knockout led to inhibition of oncogenic signaling and increased median survival compared with normal expression of both. Next steps could include evaluating pharmacological inhibitors of miR-181a and miR-181b-1.</p> <p><b>SciBX 5(34); doi:10.1038/scibx.2012.891</b> <b>Published online Aug. 30, 2012</b></p>	Patent and licensing status unavailable	<p>Fragoso, R. <i>et al. PLoS Genet.</i>; published online Aug. 9, 2012; doi:10.1371/journal.pgen.1002855 <b>Contact:</b> Chang-Zheng Chen, Stanford University School of Medicine, Stanford, Calif. e-mail: <a href="mailto:czchen@stanford.edu">czchen@stanford.edu</a></p>
Acute myelogenous leukemia (AML)	Aurora kinase A (AURKA; Aurora-A)	<p>A study in cell culture and in mice suggests AURKA inhibitors could help treat acute megakaryoblastic leukemia (AMKL), a rare form of AML. A cell-based screen for inducers of AMKL cell differentiation identified the broad kinase inhibitor dimethylfasudil. In a mouse model of AMKL, dimethylfasudil induced tumor cell differentiation and led to longer survival than vehicle. Next, AURKA was identified as the relevant target of dimethylfasudil. In the same mouse model, alisertib, a selective inhibitor of AURKA, decreased tumor burden compared with vehicle. Next steps include testing the effects of dimethylfasudil and alisertib in mouse models of myeloproliferative diseases that are driven by hyperproliferation of megakaryocytes.</p> <p>Takeda Pharmaceutical Co. Ltd.'s alisertib (MLN8237) is in Phase III testing in T cell lymphoma, Phase II testing in AML and Phase II or earlier testing in various other cancer indications.</p> <p>At least four other companies have AURKA inhibitors in clinical testing in various cancers.</p> <p><b>SciBX 5(34); doi:10.1038/scibx.2012.892</b> <b>Published online Aug. 30, 2012</b></p>	Patent application filed; available for licensing	<p>Wen, Q. <i>et al. Cell</i>; published online Aug. 3, 2012; doi:10.1016/j.cell.2012.06.032 <b>Contact:</b> John D. Crispino, Northwestern University Feinberg School of Medicine, Chicago, Ill. e-mail: <a href="mailto:j-crispino@northwestern.edu">j-crispino@northwestern.edu</a> <b>Contact:</b> Andrew M. Stern, Broad Institute of MIT and Harvard, Cambridge, Mass. e-mail: <a href="mailto:astern@broadinstitute.org">astern@broadinstitute.org</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Brain cancer	Fibroblast growth factor receptor (FGFR); FGFR3; transforming acidic coiled-coil containing protein 1 (TACC1); TACC3 (ERIC1)	<p>Mouse studies suggest FGFR kinase inhibitors could help treat a subtype of glioblastoma driven by FGFR-TACC fusion proteins. In mice, subcutaneous injection of human primary astrocytes expressing the FGFR3-TACC1 or FGFR3-TACC3 fusion proteins resulted in tumor formation, whereas injection of astrocytes expressing an inactive fusion protein, FGFR3 or TACC3 did not. In mice xenografted with FGFR3-TACC3 transformed human astrocytes, the oral FGFR inhibitor AZD4547 increased survival compared with vehicle. Next steps include running a clinical trial of an FGFR kinase inhibitor in patients with glioblastoma prescreened for FGFR-TACC fusions.</p> <p>AstraZeneca plc's AZD4547 is in Phase II testing to treat solid tumors.</p> <p>At least eight other companies have compounds targeting FGFR in Phase III to preclinical development for various cancers.</p> <p><b>SciBX 5(34); doi:10.1038/scibx.2012.893</b> Published online Aug. 30, 2012</p>	Patent application filed for diagnostic and therapeutic uses; available for licensing through Columbia Technology Ventures	<p>Singh, D. <i>et al. Science</i>; published online July 26, 2012; doi:10.1126/science.1220834 <b>Contact:</b> Antonio Iavarone, Columbia University Medical Center, New York, N.Y. e-mail: <a href="mailto:ai2102@columbia.edu">ai2102@columbia.edu</a> <b>Contact:</b> Raul Rabadan, same affiliation as above e-mail: <a href="mailto:abadan@dbmi.columbia.edu">abadan@dbmi.columbia.edu</a> <b>Contact:</b> Anna Lasorella, same affiliation as above e-mail: <a href="mailto:al2179@columbia.edu">al2179@columbia.edu</a></p>
Breast cancer	DAN domain family member 5 (DAND5; COCO)	<p>Mouse and patient sample studies suggest inhibiting COCO could help prevent breast cancer from metastasizing to the lung. In mouse models of breast cancer, vector-mediated expression of Coco promoted metastasis of breast cancer cells to the lung and induced their exit from dormancy, whereas an empty vector did not. In the mouse model, Coco promoted reactivation of metastatic cells through inhibition of bone morphogenetic protein signaling. In patients with breast cancer, a COCO gene expression signature was correlated with metastatic disease relapse to the lung but not to the bone or brain. Next steps include generating and evaluating COCO-blocking mAbs in mouse models and developing assays to identify patients with COCO-overexpressing breast carcinomas.</p> <p><b>SciBX 5(34); doi:10.1038/scibx.2012.894</b> Published online Aug. 30, 2012</p>	Patent application filed; available for licensing	<p>Gao, H. <i>et al. Cell</i>; published online Aug. 17, 2012; doi:10.1016/j.cell.2012.06.035 <b>Contact:</b> Filippo G. Giancotti, Memorial Sloan-Kettering Cancer Center, New York, N.Y. e-mail: <a href="mailto:f-giancotti@ski.mskcc.org">f-giancotti@ski.mskcc.org</a></p>
Cancer	Hypoxia-inducible factor prolyl hydroxylase 2 (EGLN1; HIF-PH2; PHD2)	<p>Mouse studies suggest PHD2 inhibitors could help improve the efficacy of chemotherapies. In <i>Phd2</i>-deficient mice with murine lung or human melanoma tumors, cisplatin or doxorubicin decreased tumor growth and led to less chemotherapy-induced renal and cardiac damage compared with what was seen in wild-type mice with tumors treated with cisplatin or doxorubicin. Additional studies in those mice showed that <i>Phd2</i> deficiency induced a protective antioxidative response to chemotherapy in normal organs but not in tumors. Ongoing work includes investigating the effects of <i>Phd2</i> deficiency in mouse models of metastatic breast cancer.</p> <p><b>SciBX 5(34); doi:10.1038/scibx.2012.895</b> Published online Aug. 30, 2012</p>	<p>Patented by the Flanders Institute for Biotechnology (VIB); available for licensing or partnering <b>Contact:</b> Barbara Leyman, Flanders Institute for Biotechnology (VIB), Flanders, Belgium e-mail: <a href="mailto:barbara.leyman@vib.be">barbara.leyman@vib.be</a></p>	<p>Leite de Oliveira, R. <i>et al. Cancer Cell</i>; published online Aug. 14, 2012; doi:10.1016/j.ccr.2012.06.028 <b>Contact:</b> Massimiliano Mazzone, Flanders Institute for Biotechnology (VIB), Flanders, Belgium e-mail: <a href="mailto:massimiliano.mazzone@vib-kuleuven.be">massimiliano.mazzone@vib-kuleuven.be</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	Integrin $\beta_3$ (GPIIIa; CD61)	Mouse studies identified a humanized single-chain antibody against an epitope of GPIIIa, dubbed GP11a, that could help prevent pulmonary metastasis in cancer. In mice injected with melanoma or Lewis lung carcinoma cells, the antibody decreased the formation of pulmonary surface nodules compared with a control antibody. In those mice, the antibody prevented metastasis by inhibiting platelet function in the tumor microenvironment. Next steps include evaluating the safety of the single-chain antibody.	Patent pending; available for licensing	Zhang, W. <i>et al. Blood</i> ; published online Aug. 9, 2012; doi:10.1182/blood-2012-04-425207 <b>Contact:</b> Wei Zhang, East China Normal University, Shanghai, China e-mail: <a href="mailto:wzhang@sat.ecnu.edu.cn">wzhang@sat.ecnu.edu.cn</a>
		<b>SciBX 5(34); doi:10.1038/scibx.2012.896</b> <b>Published online Aug. 30, 2012</b>		
Cancer	Nicotinamide phosphoribosyltransferase (NAMPT)	Cell line studies suggest carborane-based small molecule NAMPT inhibitors could help treat cancer. In human lung, breast and colon cancer cell lines, carborane-based NAMPT inhibitors had up to 10-fold greater antiproliferative activity than the parent benzoylpiperidine-based NAMPT inhibitor. Next steps include further evaluating the properties of the NAMPT inhibitors and improving their molecular structures.	Provisional patent application filed; available for licensing from the University of Missouri–Columbia <b>Contact:</b> Paul Hippenmeyer, University of Missouri–Columbia, Columbia, Mo. e-mail: <a href="mailto:hippenmeyerp@missouri.edu">hippenmeyerp@missouri.edu</a>	Lee Jr., M.W. <i>et al. J. Med. Chem.</i> ; published online Aug. 13, 2012; doi:10.1021/jm300740t <b>Contact:</b> Mark W. Lee Jr., University of Missouri–Columbia, Columbia, Mo. e-mail: <a href="mailto:leemw@missouri.edu">leemw@missouri.edu</a>
		<b>SciBX 5(34); doi:10.1038/scibx.2012.897</b> <b>Published online Aug. 30, 2012</b>		
Cancer	Wingless-type MMTV integration site family member 16B (WNT16B)	Human tissue and mouse studies suggest inhibiting stromal WNT16B could help treat cancer. In tissue samples isolated from patients with breast, ovarian or prostate cancer treated with chemotherapy, WNT16B expression was greater in the stroma than in tissues isolated from untreated patients. In patients with prostate cancer undergoing chemotherapy, increased levels of stromal WNT16B were associated with increased likelihood of cancer recurrence ( $p=0.04$ ). In xenograft mice, the genotoxic chemotherapy drug mitoxantrone decreased the size of tumors comprised of prostate cancer cells plus normal fibroblasts but not prostate cancer cells plus WNT16B-expressing fibroblasts. Next steps include developing WNT16B antibodies as adjuncts to genotoxic therapies.	Patent application filed; available for licensing	Sun, Y. <i>et al. Nat. Med.</i> ; published online Aug. 5, 2012; doi:10.1038/nm.2890 <b>Contact:</b> Peter S. Nelson, Fred Hutchinson Cancer Research Center, Seattle, Wash. e-mail: <a href="mailto:pnelson@fhcrc.org">pnelson@fhcrc.org</a>
		<b>SciBX 5(34); doi:10.1038/scibx.2012.898</b> <b>Published online Aug. 30, 2012</b>		
Melanoma	Chemerin; chemokine- like receptor 1 (CMKLR1; ChemR23)	Patient and mouse studies suggest increasing chemerin signaling could help treat melanoma. In patients with melanoma, high levels of chemerin were correlated with increased survival. In mice, chemerin-expressing melanomas showed greater NK and T cell infiltration and less growth than nonexpressing controls. In the mouse melanoma model, knocking out the chemerin receptor <i>Cmklr1</i> negated the chemoattractant's positive effects. Next steps include developing and evaluating chemerin-loaded nanoparticles in mouse cancer models.	Unpatented; licensing status not applicable	Pachynski, R.K. <i>et al. J. Exp. Med.</i> ; published online July 2, 2012; doi:10.1084/jem.20112124 <b>Contact:</b> Russell K. Pachynski, Stanford University School of Medicine, Stanford, Calif. e-mail: <a href="mailto:rkpachynski@stanford.edu">rkpachynski@stanford.edu</a>
		<b>SciBX 5(34); doi:10.1038/scibx.2012.899</b> <b>Published online Aug. 30, 2012</b>		

## This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
<b>Cardiovascular disease</b>				
Thrombosis	DNA; RNA	<p>Mouse and <i>in vitro</i> studies identified a nucleic acid-binding polymer that could help prevent thrombosis. <i>In vitro</i>, a polycationic polyamine polymer inhibited polyphosphate-mediated coagulation. In two mouse models of thrombosis, the polymer prevented thrombus formation and did not increase blood loss compared with saline. Next steps could include evaluating the lead polymer in large animal models of thrombosis.</p> <p><b>SciBX 5(34); doi:10.1038/scibx.2012.900</b> Published online Aug. 30, 2012</p>	Patent and licensing status unavailable	<p>Jain, S. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online July 25, 2012; doi:10.1073/pnas.1204928109</p> <p><b>Contact:</b> Bruce A. Sullenger, Duke University, Durham, N.C. e-mail: <a href="mailto:bruce.sullenger@duke.edu">bruce.sullenger@duke.edu</a></p>
<b>Endocrine/metabolic disease</b>				
Diabetes	Neutrophil elastase (NE; ELA-2)	<p><i>In vitro</i> and mouse studies suggest inhibiting NE could help treat diabetes. In mice fed a high-fat diet, neutrophil recruitment and NE secretion in adipose tissue and liver were greater than those in mice fed a normal diet. In mice with diet-induced obesity, an NE inhibitor or genetic knockout improved glucose tolerance. Next steps could include testing NE inhibition in additional animal models of obesity and diabetes.</p> <p>Ono Pharmaceutical Co. Ltd. markets the NE inhibitor Sivelestat to treat acute lung injury.</p> <p><b>SciBX 5(34); doi:10.1038/scibx.2012.901</b> Published online Aug. 30, 2012</p>	Patent and licensing status unavailable	<p>Talukdar, S. <i>et al. Nat. Med.</i>; published online Aug. 5, 2012; doi:10.1038/nm.2885</p> <p><b>Contact:</b> Jerrold M. Olefsky, University of California, San Diego, La Jolla, Calif. e-mail: <a href="mailto:jolefsky@ucsd.edu">jolefsky@ucsd.edu</a></p>
<b>Infectious disease</b>				
Influenza virus	Influenza A virus hemagglutinin (HA); influenza B HA	<p><i>In vitro</i> and mouse studies suggest human mAbs could be used to treat or prevent influenza A and influenza B virus infections. Screening of a combinatorial display library of human B cells from volunteers vaccinated with a seasonal influenza vaccine identified an antibody that bound HA from group 1 and group 2 influenza A virus and both lineages of influenza B virus. Mice receiving the antibody were protected from challenge with H1N1 or H3N2 influenza A virus and both lineages of influenza B virus. Next steps include evaluating the safety of the antibody.</p> <p><b>SciBX 5(34); doi:10.1038/scibx.2012.902</b> Published online Aug. 30, 2012</p>	Patent application filed; unavailable for licensing	<p>Dreyfus, C. <i>et al. Science</i>; published online Aug. 9, 2012; doi:10.1126/science.1222908</p> <p><b>Contact:</b> Jaap Goudsmit, Crucell Vaccine Institute, Leiden, the Netherlands e-mail: <a href="mailto:jaap.goudsmit@crucell.com">jaap.goudsmit@crucell.com</a></p>
<b>Neurology</b>				
Alzheimer's disease (AD)	GABA <sub>A</sub> receptor	<p>Cell culture and mouse studies suggest compounds mimicking both nitric oxide (NO) and <math>\gamma</math>-aminobutyric acid (GABA) could help treat AD. In an assay of excitation-induced cell death, GABA<sub>A</sub> receptor agonists with an NO-mimicking moiety increased cell survival compared with conventional GABA<sub>A</sub> receptor agonists. In a mouse model of drug-induced memory loss, the compounds increased memory recovery compared with vehicle. Next steps include development of a related compound in the same series.</p> <p><b>SciBX 5(34); doi:10.1038/scibx.2012.903</b> Published online Aug. 30, 2012</p>	Patents pending and issued; licensed to sGC Pharma Inc.	<p>Qin, Z. <i>et al. J. Med. Chem.</i>; published online July 10, 2012; doi:10.1021/jm300353r</p> <p><b>Contact:</b> Gregory R.J. Thatcher, University of Illinois at Chicago College of Pharmacy, Chicago, Ill. e-mail: <a href="mailto:thatcher@uic.edu">thatcher@uic.edu</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Alzheimer's disease (AD); amyotrophic lateral sclerosis (ALS); Parkinson's disease (PD); Huntington's disease (HD)	Neural precursor cell expressed developmentally downregulated 4 E3 ubiquitin protein ligase (NEDD4; NEDD4-1)	<i>In vitro</i> and mouse studies suggest inhibiting NEDD4-1 could help treat neurodegenerative diseases. In brain and spinal cord tissue samples from patients with neurodegenerative diseases, NEDD4-1 levels were greater than those in samples from healthy controls. In cultured rat primary cortical neurons and in mice, exposure to neurotoxins increased Nedd4-1 levels compared with no exposure. In mouse neuroblastoma cells, inhibiting Nedd4-1 signaling decreased neurotoxin-induced cell death compared with no inhibition. Next steps could include developing a NEDD4-1 inhibitor.  <b>SciBX 5(34); doi:10.1038/scibx.2012.904</b> <b>Published online Aug. 30, 2012</b>	Unpatented; licensing status not applicable	Kwak, Y.-D. <i>et al. J. Neurosci.</i> ; published online Aug. 8, 2012; doi:10.1523/JNEUROSCI.1836-12.2012 <b>Contact:</b> Francesca-Fang Liao, The University of Tennessee Health Science Center, Memphis, Tenn. e-mail: <a href="mailto:fliao@uthsc.edu">fliao@uthsc.edu</a>
Multiple sclerosis (MS)	Galectin-1 (LGALS1)	<i>In vitro</i> and mouse studies suggest LGALS1 could help treat MS. In a mouse model of experimental autoimmune encephalitis (EAE), knocking out <i>Lgals1</i> induced activation of proinflammatory microglia and increased axonal loss compared with normal <i>Lgals1</i> expression. In <i>Lgals1</i> knockout mice with EAE, <i>Lgals1</i> infusion or adoptive transfer of <i>Lgals1</i> -expressing astrocytes decreased microglia activation and disease severity compared with vehicle infusion or transfer of <i>Lgals1</i> -deficient astrocytes. Next steps include developing a small molecule that mimics LGALS1's effects.  <b>SciBX 5(34); doi:10.1038/scibx.2012.905</b> <b>Published online Aug. 30, 2012</b>	Unpatented; licensing status not applicable	Starossom, S.C. <i>et al. Immunity</i> ; published online Aug. 9, 2012; doi:10.1016/j.immuni.2012.05.023 <b>Contact:</b> Gabriel A. Rabinovich, National Scientific and Technical Research Council, Buenos Aires, Argentina e-mail: <a href="mailto:gabyrabi@gmail.com">gabyrabi@gmail.com</a> <b>Contact:</b> Samia J. Khoury, Harvard Medical School, Boston, Mass. e-mail: <a href="mailto:skhoury@rics.bwh.harvard.edu">skhoury@rics.bwh.harvard.edu</a>
Stroke	Ephrin-A5 (EFNA5; AL-1)	Mouse studies suggest inhibiting EFNA5 could help promote recovery from stroke. In mice with stroke-induced infarcts in the forelimb motor cortex, post-injury blockade of <i>Efna5</i> signaling in the infarct region increased axonal sprouting and recovery of forelimb function compared with no blockade. Next steps include developing biopolymer hydrogels to deliver biologics to the stroke cavity to promote recovery.  <b>SciBX 5(34); doi:10.1038/scibx.2012.906</b> <b>Published online Aug. 30, 2012</b>	Patented; licensed to an undisclosed company	Overman, J.J. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 25, 2012; doi:10.1073/pnas.1204386109 <b>Contact:</b> S. Thomas Carmichael, University of California, Los Angeles, Calif. e-mail: <a href="mailto:scarmichael@mednet.ucla.edu">scarmichael@mednet.ucla.edu</a>
Stroke	Unknown	Rat studies suggest 3-pentylbenzo[c]thiophen-1(3 <i>H</i> )-one could help treat stroke. In rats, oral pretreatment with the compound decreased stroke-induced neurological deficits and infarct size compared with saline pretreatment. Next steps could include testing the compound in large animal models of stroke.  <b>SciBX 5(34); doi:10.1038/scibx.2012.907</b> <b>Published online Aug. 30, 2012</b>	Patent and licensing status unavailable	Wu, J. <i>et al. J. Med. Chem.</i> ; published online July 24, 2012; doi:10.1021/jm300681r <b>Contact:</b> Yihua Zhang, China Pharmaceutical University, Nanjing, China e-mail: <a href="mailto:zyhtgd@hotmail.com">zyhtgd@hotmail.com</a> <b>Contact:</b> Hui Ji, same affiliation as above e-mail: <a href="mailto:huijicpu@163.com">huijicpu@163.com</a>

## This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
<b>Ophthalmic disease</b>				
Retinitis	Receptor-interacting serine-threonine kinase 1 (RIPK1; RIP1); RIPK3 (RIP3)	<p>Mouse studies suggest inhibiting RIPK signaling could help treat retinitis pigmentosa. In a mouse model of retinitis pigmentosa, <i>Rip3</i> knockout decreased cone photoreceptor death compared with normal <i>Rip3</i> expression. Also in those mice, a small molecule RIP1 inhibitor decreased cone photoreceptor cell death compared with vehicle. Next steps include seeking an industry partner or funding to carry out IND-enabling studies.</p> <p>SciBX 5(34); doi:10.1038/scibx.2012.908 Published online Aug. 30, 2012</p>	<p>Patent application filed covering use in indications related to retinal degeneration; available for licensing from the Massachusetts Eye and Ear Infirmary Contact: Ojas Mehta, Massachusetts Eye and Ear Infirmary, Boston, Mass. e-mail: <a href="mailto:ojas_mehta@meei.harvard.edu">ojas_mehta@meei.harvard.edu</a></p>	<p>Murakami, Y. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Aug. 20, 2012; doi:10.1073/pnas.1206937109 Contact: Demetrios G. Vavvas, Harvard Medical School, Boston, Mass. e-mail: <a href="mailto:demetrios_vavvas@meei.harvard.edu">demetrios_vavvas@meei.harvard.edu</a></p>

## This week in techniques

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

This week in techniques includes findings about research tools, disease models and manufacturing processes that have the potential to enable or improve all stages of drug discovery and development.

Approach	Summary	Licensing status	Publication and contact information
<b>Assays &amp; screens</b>			
Prenatal genome sequencing	A study in pregnant women suggests fetal whole-genome sequencing could help detect chromosomal abnormalities. In blood from two pregnant women, a DNA isolation and enrichment procedure allowed the sequencing of the complete fetal genome, leading to the detection of a 2.85 Mb deletion associated with DiGeorge syndrome. Next steps could include development of screening procedures to detect the presence of hereditary disease alleles in fetal DNA circulating in maternal blood.  <b>SciBX 5(34); doi:10.1038/scibx.2012.909</b> <b>Published online Aug. 30, 2012</b>	Patent and licensing status unavailable	Fan, H.C. <i>et al. Nature</i> ; published online July 4, 2012; doi:10.1038/nature11251 <b>Contact:</b> Stephen R. Quake, Stanford University, Stanford, Calif. e-mail: <a href="mailto:quake@stanford.edu">quake@stanford.edu</a>
<b>Disease models</b>			
Transgenic mouse model for schizophrenia	Transgenic mice with astrocyte-specific overexpression of human heme oxygenase decycling 1 (HMOX1; HO-1; Hsp32) could be a useful model for schizophrenia and other neurodevelopmental and neurodegenerative diseases. In behavioral assays, the mice showed multiple schizophrenia-like behaviors including hyperlocomotion and impaired prepulse inhibition. Brain tissue samples showed multiple neuropathologies including altered hippocampal cytoarchitecture, subcortical oxidative stress and mitochondrial damage. Next steps include further characterizing the phenotype of the mice and using the model to evaluate the effects of a brain-permeable HO-1 inhibitor from Osta Biotechnologies Inc. Osta's OB-28 HO-1 inhibitor is in preclinical development for Alzheimer's disease (AD).  <b>SciBX 5(34); doi:10.1038/scibx.2012.910</b> <b>Published online Aug. 30, 2012</b>	Patent application filed; available for licensing	Song, W. <i>et al. J. Neurosci.</i> ; published online Aug. 8, 2012; doi:10.1523/JNEUROSCI.6469-11.2012 <b>Contact:</b> Hyman M. Schipper, Jewish General Hospital, Montreal, Quebec, Canada e-mail: <a href="mailto:hyman.schipper@mcgill.ca">hyman.schipper@mcgill.ca</a>
<b>Drug delivery</b>			
Nonviral DNA nanoparticles for ocular gene therapy to treat Stargardt's disease	Mouse studies suggest nonviral DNA nanoparticles could be used for ocular gene therapy to help treat Stargardt's disease, a retinal degenerative disorder caused by mutations in <i>ATP-binding cassette sub-family A member 4</i> ( <i>ABCA4</i> ; <i>ABCR</i> ). In <i>Abca4</i> -deficient mice, nonviral DNA nanoparticles carrying human <i>ABCA4</i> cDNA vectors and a photoreceptor-targeting promoter induced <i>ABCA4</i> expression and decreased disease symptoms compared with saline. Next steps include testing the technology for delivery of a larger gene associated with vision disorders.  <b>SciBX 5(34); doi:10.1038/scibx.2012.911</b> <b>Published online Aug. 30, 2012</b>	U.S. utility patent application filed; available for licensing	Han, Z. <i>et al. J. Clin. Invest.</i> ; published online Aug. 13, 2012; doi:10.1172/JCI64833 <b>Contact:</b> Muna I. Naash, The University of Oklahoma Health Sciences Center, Oklahoma City, Okla. e-mail: <a href="mailto:muna-naash@ouhsc.edu">muna-naash@ouhsc.edu</a>
<b>Drug platforms</b>			
Anandamide–small interfering RNA conjugates for targeting neuronal and immune cells	Anandamide-siRNA conjugates that silence gene expression in neuronal and immune cells could be useful for treating immunological and neurological diseases. The conjugates were generated by linking alkyne-modified RNA with 3'-anandamide and produced at high yield and purity. In a rat basophilic leukemia cell line that models neuronal cells and astrocytes, the anandamide-siRNA conjugates decreased expression of a targeted gene compared with unmodified RNA duplexes and anandamide-modified scrambled siRNA. Next steps could include determining the tissue distribution of the anandamide-siRNA conjugates in animal models.  <b>SciBX 5(34); doi:10.1038/scibx.2012.912</b> <b>Published online Aug. 30, 2012</b>	Patent and licensing status unavailable	Willibald, J. <i>et al. J. Am. Chem. Soc.</i> ; published online July 19, 2012; doi:10.1021/ja303251f <b>Contact:</b> Thomas Carell, Ludwig Maximilian University of Munich, Munich, Germany e-mail: <a href="mailto:thomas.carell@cup.uni-muenchen.de">thomas.carell@cup.uni-muenchen.de</a>

## This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
<b>Markers</b>	<p>A human genetic study suggests mutations in the sperm of older fathers could influence disease in their offspring. Whole-genome sequencing of 78 individuals and their parents identified an average of 63.2 new mutations per person per generation. The number of mutations was higher in individuals sired by older fathers than in individuals with younger fathers. Next steps include determining the role of these new mutations in diseases such as autism spectrum disorder (ASD) and schizophrenia. (see <b>deCode-ing autism, page 6</b>)</p>	<p>Unpatented; licensing status not applicable</p>	<p>Kong, A. <i>et al. Nature</i>; published online Aug. 22, 2012; doi:10.1038/nature11396  <b>Contact:</b> Kári Stefánsson, deCode genetics ehf, Reykjavik, Iceland  e-mail: <a href="mailto:kstefans@decode.is">kstefans@decode.is</a></p>
<p>Mutations linked to paternal age</p>	<p>SciBX 5(34); doi:10.1038/scibx.2012.913  Published online Aug. 30, 2012</p>		

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