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RON's new role

By *Chris Cain, Senior Writer*

A Utah team has shown that inhibiting the kinase RON could fight cancer metastasis by stimulating an antitumor immune response.¹ The findings build a case for directed drug discovery efforts against the target, which has long been neglected in favor of its more famous relative, MET.

In the last few years, two drugs have been approved that inhibit MET (c-Met proto-oncogene; HGFR): Xalkori crizotinib from **Pfizer Inc.** to treat EML4-ALK oncogenic fusion protein-positive lung cancer and Cometriq cabozantinib from **Exelixis Inc.** to treat medullary thyroid cancer.

At least 14 additional companies are developing small molecules or antibodies against the target.

RON (macrophage stimulating 1 receptor c-Met-related tyrosine kinase; MST1R; CD136) shares considerable structural and sequence homology with MET but nevertheless has not been as closely scrutinized as a cancer target. Exelixis CSO and EVP of discovery research Peter Lamb told *SciBX* that the choice to prioritize MET was based on the vast amount of preclinical evidence that supports its role in driving cancer.

“When we started out on the work that led to the development of cabozantinib, there was evidence that *MET* expression, *MET* amplification and upregulation of its ligand, hepatocyte growth factor/scatter factor (HGF/SF), contributed to cancer metastasis and had effects on survival,” he said. “It was simply a much more intensively studied target, and there was much less data on RON, so it was not a difficult choice to focus on MET.”

Indeed, researchers initially studied RON primarily for its role in regulating inflammation. Only recently has overexpression of RON or its ligand, macrophage stimulating protein (MST1; MSP), been found in many malignancies.² The only disclosed anti-RON-specific program in development is narnatumab (IMC-RON8), an anti-RON mAb from **Eli Lilly and Co.** that is in Phase I trials for solid tumors.

In 2010, **Aveo Pharmaceuticals Inc.** and **Johnson & Johnson** partnered to develop anti-RON mAbs,³ but that partnership was terminated late last year for undisclosed reasons.

Aveo, J&J and Lilly declined interview requests.

Some small molecule MET inhibitors, including cabozantinib, have some inhibitory activity against RON because of homology between the proteins' kinase domains. However, Lamb said Exelixis was agnostic about the activity of cabozantinib against RON during the development of the compound and does not have data on whether RON is a clinically relevant target of cabozantinib.

Now, researchers at the **Huntsman Cancer Institute at The University of Utah** have linked the inflammatory- and cancer-associated functions of RON and provided new evidence to support the rational design of inhibitors of this target.

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PO Box 1246
San Carlos, CA 94070-1246
T: +1 650 595 5333Chadds Ford
223 Wilmington-West Chester Pike
Chadds Ford, PA 19317
T: +1 610 558 1873Chicago
20 N. Wacker Drive, Suite 1465
Chicago, IL 60606-2902
T: +1 312 755 0798Oxford
287 Banbury Road
Oxford OX4 7JA
United Kingdom
T: +44 (0)18 6551 2184Washington, DC
2008 Q Street, NW, Suite 100
Washington, DC 20009
T: +1 202 462 9582**Nature Publishing Group**New York
75 Varick Street, 9th Floor
New York, NY 10013-1917
T: +1 212 726 9200London
The Macmillan Building
4 Crinan Street
London N1 9XW
United Kingdom
T: +44 (0)20 7833 4000Tokyo
Chiyoda Building 6F
2-37 Ichigayatamachi
Shinjuku-ku, Tokyo 162-0843
Japan
T: +81 3 3267 8751

SciBX is produced by BioCentury Publications, Inc. and Nature Publishing Group Joint Steering Committee: Karen Bernstein, Ph.D., Chairman & Editor-in-Chief, BioCentury; David Flores, President & CEO, BioCentury; Bennet Weintraub, Finance Director, BioCentury; Steven Inchcoombe, Managing Director, Nature Publishing Group; Peter Collins, Ph.D., Publishing Director, NPG; Christoph Hesselmann, Ph.D., Chief Financial Officer, NPG.

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The group built on 2007 postdoctoral work by Alana Welm and J. Michael Bishop at the **University of California, San Francisco**. Welm and Bishop showed that overexpression of RON or MSP was linked to poor prognosis in patients with breast cancer and further demonstrated that increased MSP expression could drive metastasis in a mouse model for breast cancer.⁴

Welm is now an assistant professor in the Department of Oncological Sciences at Huntsman and is corresponding author of the new study. Bishop is chancellor emeritus of UCSF and remains a professor in the Department of Microbiology and Immunology at UCSF.

To better understand how RON and MSP drive metastasis—and to explore the therapeutic potential of RON inhibition—her team implanted MSP-overexpressing mouse breast cancer cells into either wild-type mice or knockout mice in which Ron's intracellular kinase domain had been deleted. In knockout mice lacking Ron activity, compared with in wild-type mice, spontaneous lung metastases were dramatically decreased and survival was significantly prolonged ($p < 0.05$).

Importantly, the model used tumor cells implanted into genetically matched immunocompetent hosts. Unlike commonly used immunodeficient xenograft mouse models, this approach allowed the researchers to analyze the effect of the immune system on tumor growth and metastasis.

Additionally, more CD8⁺ T cells were associated with tumors in the *Ron* kinase domain knockout mice than in the wild-type mice. These T cells and macrophages produced higher levels of proinflammatory tumor necrosis factor- α (TNF- α), suggesting the reduction of metastasis was caused by an antitumor immune response.

Indeed, antibody-mediated depletion of CD8⁺ T cells increased metastasis in the knockout mice, whereas transplantation of CD8⁺ T cells

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from the knockout mice into immunodeficient mice reduced metastasis.

Finally, to test the therapeutic relevance of inhibiting RON, the team turned to ASLAN002 (formerly BMS-777607), a small molecule RON and MET inhibitor from **Aslan Pharmaceuticals Pte. Ltd.** Mice receiving ASLAN002 before tumor cell injection had decreased metastasis compared with controls given vehicle. This effect was prevented by a CD8⁺ T cell-depleting antibody.

In mice in which metastasis was allowed to begin prior to treatment, a model with more clinical relevance, ASLAN002 decreased metastatic outgrowth about fourfold compared with vehicle.

Results were published in *Cancer Discovery*.

Humanizing RON

Welm told *SciBX* that the results will change the way RON is viewed as a cancer target.

“Inhibiting Ron kinase activity allows an effective antitumor CD8⁺ T cell response, which inhibits outgrowth of seeded metastatic tumor colonies. This dramatically changes the way that we think about RON inhibitors in the clinic,” she said. “Currently, RON inhibitors are explored as agents that might directly shrink tumor growth. Our work shows that RON inhibitors may actually work as bona fide immune-stimulating agents, much like ipilimumab. This could impact clinical development of RON inhibitors, including rational clinical trial design, patient selection and monitoring of clinical efficacy.”

Bristol-Myers Squibb Co. markets Yervoy ipilimumab to treat metastatic melanoma. The human mAb promotes cytotoxic T cell–mediated killing of tumor cells by blocking the CTLA-4 (CD152) receptor.

Welm said understanding the mechanism underlying RON inhibition is crucial for properly evaluating compounds. For example, she noted that blocking RON activity did not affect primary tumor growth in her study, and thus the approach could appear ineffective based on traditional response rate criteria.

As a result, measuring immune response markers, metastatic growth and overall survival could be important in evaluating the effects of a RON inhibitor. She added that expression of MSP could provide a tumor biomarker for sensitivity to RON inhibition.

Indeed, Welm is collaborating with Aslan to continue study of ASLAN002, which is currently being tested in a Phase I trial for solid tumors. She told *SciBX* that ASLAN002 is the only small molecule she knows of that is more selective for RON than for MET and is being tested clinically.

Aslan CSO and cofounder Mark McHale told *SciBX* that the company licensed the compound from Bristol-Myers for its anti-MET activity but is exploring new uses based on Welm’s preclinical results.

“Based on Alana’s work and work by our own investigators, we are actively looking at testing the compound in patients with bone metastases and will be looking for biomarkers such as macrophage activation and TNF- α expression,” he said. McHale noted that inhibiting MET signaling also has been shown to have antimetastatic effects through distinct mechanisms of action.

Exelixis’ Lamb said the Utah team’s studies suggest it may be worthwhile to look at markers such as tumor-infiltrating lymphocytes and macrophages if patient biopsy samples are available from trials of cabozantinib or other MET and RON inhibitors.

Exelixis’ cabozantinib is in two Phase III trials to treat prostate cancer,

both of which are examining bone scan response as a secondary endpoint.

He added that the results increase the attractiveness of RON as a drug target and emphasized that the work reinforces scientific rationale for testing whether cabozantinib can prevent bone metastases.

Ravi Salgia, professor of medicine, pathology and dermatology at **The University of Chicago**, said Welm’s results, along with work from his own lab showing RON plays a role in gastroesophageal and lung cancers,^{5,6} argue for a renewed focus on RON as a drug target.

“RON is no longer the little brother of MET; it has come into its own,” he said. “We are absolutely convinced RON is a therapeutic target in and of itself.”

Salgia is most interested in seeing RON inhibition combined with cytotoxic chemotherapies. Welm is planning such studies, and McHale said Aslan is considering testing ASLAN002 in combination with other compounds including its pan-epidermal growth factor receptor (EGFR) inhibitor, ASLAN001.

Sandra Demaria, associate professor of pathology at the **New York University Langone Medical Center**, agreed that combination studies are a key next step and said that this study published in *Cancer Discovery* provides another example in which a therapeutic strategy that was not designed to be an immunotherapy in fact drives important antitumor immune responses.

As another recent example, she pointed to a 2011 study that showed that the tyrosine kinase inhibitor Gleevec imatinib unexpectedly induced antitumor immune responses in gastrointestinal stromal tumors (GISTs) by reducing expression of *indoleamine 2,3-dioxygenase* (INDO; IDO).⁷

Gleevec is marketed by **Novartis AG** to treat multiple cancers.

Demaria added that the new findings provide additional evidence that immunocompetent mouse models are critical to understanding how therapeutics work. “*In vivo* everybody has relied on immunodeficient mice, but this really brings a biased view of how therapeutics act,” she said.

The findings of the Utah team are not patented.

Cain, C. *SciBX* 6(21); doi:10.1038/scibx.2013.507

Published online May 30, 2013

REFERENCES

1. Eyob, H. *et al. Cancer Discov.*; published online April 23, 2013; doi:10.1158/2159-8290.CD-12-0480
Contact: Alana L. Welm, Huntsman Cancer Institute at The University of Utah, Salt Lake City, Utah
e-mail: alana.welm@hci.utah.edu
2. Wagh, P.K. *et al. Adv. Cancer Res.* **100**, 1–33 (2008)
3. Flanagan, M. *BioCentury* **19**(24), A6–A7; June 6, 2011
4. Welm, A.L. *et al. Proc. Natl. Acad. Sci. USA* **104**, 7570–7575 (2007)
5. Catenacci, D.V.T. *et al. Cancer Biol. Ther.* **12**, 9–46 (2011)
6. Kanteti, R. *et al. Genes Chromosomes Cancer* **51**, 841–851 (2012)
7. Balachandran, V.P. *et al. Nat. Med.* **17**, 1094–1100 (2011)

COMPANIES AND INSTITUTIONS MENTIONED

Aslan Pharmaceuticals Pte. Ltd., Singapore
Aveo Pharmaceuticals Inc. (NASDAQ:AVEO), Cambridge, Mass.
Bristol-Myers Squibb Co. (NYSE:BMJ), New York, N.Y.
Eli Lilly and Co. (NYSE:LLY), Indianapolis, Ind.
Exelixis Inc. (NASDAQ:EXEL), South San Francisco, Calif.
Huntsman Cancer Institute at The University of Utah, Salt Lake City, Utah
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
New York University Langone Medical Center, New York, N.Y.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Pfizer Inc. (NYSE:PFE), New York, N.Y.
University of California, San Francisco, Calif.
The University of Chicago, Chicago, Ill.

Doubts about Targretin in AD

By Lev Osherovich, Senior Writer

Four independent academic teams have called into question¹⁻⁴ some conclusions from a 2012 paper by **Case Western Reserve University School of Medicine** researchers that suggested the cancer drug Targretin bexarotene could be useful for treating Alzheimer's disease.⁵ The new studies failed to replicate some aspects of the original work, but the main conclusions—that Targretin reduces levels of soluble β -amyloid and improves cognitive function in mice—appear to stand.

A clearer picture should emerge in early 2014, when researchers from the **Cleveland Clinic Lou Ruvo Center for Brain Health** are expected to report findings from a Phase II trial of bexarotene to treat AD.

Targretin is marketed by **Eisai Co. Ltd.** to treat cutaneous T cell lymphoma (CTCL) and went off patent last year. The compound is an agonist of retinoid X receptor (RXR), a transcription factor that controls expression of proteins involved in lipid transport in the brain. Defects in lipid transport contribute to AD.

In the original study, a team led by Case Western professor of neurosciences and neurology Gary Landreth examined the effect of agonizing RXR on β -amyloid ($A\beta$) clearance and cognition in a mouse model for AD.

In one commonly used mouse model for AD, Targretin led to higher levels of apolipoprotein E (ApoE), a lipid-ferrying protein that counteracts $A\beta$ accumulation, than did vehicle. Targretin-treated mice also had lower levels of soluble $A\beta$ and $A\beta$ plaques in the brain and performed better in tests of cognition and memory than vehicle-treated controls.

Because of the striking effect of Targretin on $A\beta$ plaques—about a 75% decrease compared with controls—-independent academic teams at the **University of Pittsburgh**, the **University of Florida College of Medicine**, the **Catholic University Leuven** and a consortium of researchers at **The University of Chicago**, **Harvard Medical School**, the **University of Tuebingen**, the **Northwestern University Feinberg School of Medicine** and the **Washington University in St. Louis School of Medicine** tried to replicate Landreth's study.

In each case, the teams examined the effects of Targretin or generic formulations of bexarotene in a wide range of mouse models for AD as well as in dogs.

None of the four teams saw a statistically significant decrease in $A\beta$ plaque levels after treatment. Two of the four teams found that bexarotene decreased levels of soluble $A\beta$ in cerebrospinal fluid or interstitial fluid compared with vehicle, whereas the other two teams saw no statistically significant change in soluble $A\beta$.

The Leuven team, headed by Bart De Strooper, professor of human genetics, tested the effects of bexarotene on cognition and memory and saw that treated animals had modestly better performance in an assay of social memory compared with animals given vehicle.

De Strooper noted in his technical comment in *Science* that the drug's toxicity led to behavioral abnormalities that made cognitive assays difficult to interpret.

In contrast, a team co-led by Radosveta Koldamova and Iliya Lefterov, both associate professors of environmental and occupational health at the University of Pittsburgh, showed that bexarotene improved memory and cognition in line with Landreth's findings.

Results from all four studies were reported as non-peer reviewed technical comments in *Science*.

Formulation fulmination

In a published response to the four technical comments,⁶ Landreth's team suggested that differences in drug formulation could explain the discrepancy between the original study and the efforts to replicate it.

For example, Landreth's team noted that the toxicity and lack of efficacy seen by De Strooper's team could be due to the use of a different vehicle. Landreth's team used off-the-shelf Targretin capsules solubilized in water. De Strooper's team used cyclodextrin-based solvents.

Of the four groups, the University of Pittsburgh team used the closest formulation to the one in Landreth's original paper. That team saw the most pronounced results with the drug.

The findings highlight the difficulty in consistently conducting seemingly similar assays in different laboratories. For example, the fact that two of the new teams saw reductions in soluble $A\beta$ and the other two teams did not suggests that subtle methodological differences can lead to highly varied results.

There is little consensus about how changes in biomarkers such as soluble $A\beta$ or plaque levels in mice relate to human disease. Likewise, it is unclear whether improving mouse learning and memory would lead to meaningful improvements in functional and cognitive endpoints in humans, the clinical standard for AD therapy.

Landreth cofounded **ReXceptor Inc.** to develop brain-penetrating formulations of bexarotene to treat AD. The company plans to start a Phase Ib trial in healthy volunteers.

"The trial will use the FDA-approved dose of the drug, administered exactly as described on the Eisai label," said Landreth.

The Cleveland Clinic is running a Phase II trial of bexarotene. It has not been disclosed how that formulation differs from ReXceptor's.

Osherovich, L. *SciBX* 6(21); doi:10.1038/scibx.2013.508
Published online May 30, 2013

REFERENCES

1. Fitz, N.F. *et al. Science*; published online May 24, 2013; doi:10.1126/science.1235809
Contact: Radosveta Koldamova, University of Pittsburgh, Pittsburgh, Pa.
e-mail: radak@pitt.edu
Contact: Iliya Lefterov, same affiliation as above
e-mail: ilijal@pitt.edu
2. Price, A.R. *et al. Science*; published online May 24, 2013; doi:10.1126/science.1234089
Contact: Kevin M. Felsenstein, University of Florida College of Medicine, Gainesville, Fla.
e-mail: kfelsenstein0@ufl.edu

3. Tesseur, I. *et al. Science*; published online May 24, 2013; doi:10.1126/science.1233937
Contact: Bart De Strooper, Catholic University Leuven, Leuven, Belgium
 e-mail: bart.destrooper@cme.vib-kuleuven.be
4. Veeraraghavalu, K. *et al. Science*; published online May 24, 2013; doi:10.1126/science.1235505
Contact: Mathias Jucker, University of Tuebingen, Tuebingen, Germany
 e-mail: mathias.jucker@uni-tuebingen.de
Contact: David M. Holtzman, Washington University in St. Louis School of Medicine, St. Louis, Mo.
 e-mail: holtzman@neuro.wustl.edu
Contact: Rudolph E. Tanzi, Harvard Medical School, Cambridge, Mass.
 e-mail: tanzi@helix.mgh.harvard.edu
Contact: Robert Vassar, Northwestern University Feinberg School of Medicine, Chicago, Ill.
 e-mail: r-vassar@northwestern.edu
Contact: Sangram S. Sisodia, The University of Chicago, Chicago, Ill.
 e-mail: ssisodia@bsd.uchicago.edu

5. Cramer, P.E. *et al. Science* **335**, 1503–1506 (2012)
6. Landreth, G.E. *et al. Science*; published online May 24, 2013; doi:10.1126/science.1234114
Contact: Gary E. Landreth, Case Western Reserve University School of Medicine, Cleveland, Ohio
 e-mail: gel2@case.edu

COMPANIES AND INSTITUTIONS MENTIONED

Case Western Reserve University School of Medicine, Cleveland, Ohio
Catholic University Leuven, Leuven, Belgium
Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, Nev.
Eisai Co. Ltd. (Tokyo:4523; Osaka:4523), Tokyo, Japan
Harvard Medical School, Boston, Mass.
Northwestern University Feinberg School of Medicine, Chicago, Ill.
ReXceptor Inc., Cleveland, Ohio
The University of Chicago, Chicago, Ill.
University of Florida College of Medicine, Gainesville, Fla.
University of Pittsburgh, Pittsburgh, Pa.
University of Tuebingen, Tuebingen, Germany
Washington University in St. Louis School of Medicine, St. Louis, Mo.

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Synthetic influenza seeds

By Amy Donner, Senior Editor

An international team led by **Novartis AG** and the **J. Craig Venter Institute** has improved the speed and accuracy of seed influenza virus production for large-scale vaccine manufacture.¹ Adoption of the platform could shave weeks off the time needed to generate vaccine in response to a pandemic.

Influenza vaccines typically are developed seasonally from seed viruses representing the year's prevalent or virulent strains. The stocks of seed viruses are produced at centralized facilities working in concert with the **World Health Organization** and are distributed for vaccine manufacturing via a complex process involving multiple institutions and shipping steps.

The slow distribution of seed virus contributes to the overall inefficient production of influenza vaccine in response to pandemics.

The six-month response time to the 2009 H1N1 influenza virus—spanning the whole cycle from recognition of the pandemic to vaccine availability—is the best on record, but the swine flu vaccine was still only available to the public after the pandemic had actually peaked.

To accelerate seed virus production, a team led by Philip Dormitzer, Rino Rappuoli and J. Craig Venter took on two major technical barriers to the production of synthetic seed virus: the speed and accuracy of influenza genome synthesis.

Dormitzer is head of virology and research in the U.S. at Novartis' Vaccines and Diagnostics unit. Rappuoli is global head of vaccine research at Novartis Vaccines and Diagnostics, and Venter is founder, chairman and CEO of the J. Craig Venter Institute (JCVI) and founder of **Synthetic Genomics Inc.**

The collaborators started by adapting protocols developed by JCVI for synthesizing the mouse mitochondrial genome² to the synthesis of genes encoding influenza antigens.

The synthetic process involved production of overlapping and complementary oligonucleotides representing the complete coding sequences for the 1.7 kb influenza A virus hemagglutinin (HA) and influenza B virus HA or the 1.5 kb neuraminidase (NA), which are viral surface proteins that serve as the basis of most approved flu vaccines. Assembly of the segments did not require cloning or sequencing steps.

Initial attempts to assemble viral gene segments based on the mitochondrial protocol yielded only about 3% of product with the right sequence. Thus, the team worked to improve the assembly protocol by extending the overlap between complementary oligonucleotides, adding an enzymatic error correction step and increasing the number of oligonucleotides assembled at once.

The changes turned the original stepwise series of subassembly processes into a one-step process that produced gene segments with the correct sequence more than 80% of the time.

The synthetic genomic segments were combined with elements necessary for expression of HA or NA and introduced into Madin-Darby canine kidney (MDCK) cells, a cell line approved for vaccine manufacture.

The seed virus—a recombinant construct containing the viral gene segments with their expression elements in a viral vector—was recovered

from cells with up to 1,000-fold higher efficiency than that for a previously described system³ and up to 9.9-fold greater HA yield than that for reference strains.

Improving the yield of HA and efficiency of recovery can also shorten the overall time to vaccine generation. Similar results were obtained with at least six influenza strains.

In a simulated pandemic, the synthetic approach yielded seed virus in four days and four hours from receipt of sequence. In contrast, during the 2009 pandemic it took 36 days from identification of the virus to the manufacturers receiving the seed virus, according to a report from the **Center for Infectious Disease Research & Policy (CIDRAP)** at the **University of Minnesota**.⁴

Importantly, when tested in ferrets, the synthetic seed vaccine produced in the simulated pandemic was equivalent to conventional vaccines for antigenicity or induction of an immune response.

Results were published in *Science Translational Medicine*. The team included researchers from Synthetic Genomics, the **Biomedical Advanced Research and Development Authority** and the **Philipps University of Marburg**.

According to Dormitzer, Novartis and the Venter groups are working to automate additional seed-generation steps. Improvements in logistical components of the process—for example, reducing shipping time of samples between collaborating institutions—could further speed up vaccine generation.

“Rapid synthesis of a seed virus is important, and this is probably how it should be done.”

**—Steffen Mueller,
Codagenix Inc.**

One of many steps

Although the synthetic seed virus production platform provides time savings for one step in vaccine production, other time-consuming steps, particularly growing seed virus for large-scale manufacture of vaccine, have a greater impact on the overall response time to a pandemic.

Indeed, the production of sufficient quantities of vaccine from the seed virus generally represents the rate-limiting step, so the rapid method for generating seed virus will still be followed by the slowest, most time-consuming step of the process.

According to Steffen Mueller, president and CSO of **Codagenix Inc.**, “Rapid synthesis of a seed virus is important, and this is probably how it should be done. But with current antigens you need a lot to get an immune response, so you need to grow a lot of virus. Growing massive amounts of virus takes time. This synthetic approach will only shave off two to three weeks of a six-month process.”

One source of inefficiency is reliance upon growing the virus in embryonated chicken eggs with subsequent purification of the vaccine antigen for large-scale vaccine production.

Some companies have received approval for alternative manufacturing platforms that use cell-based systems.

Novartis, which already is implementing MDCK cell lines for influenza vaccine production, is now seeking approval to use the same cells to produce synthetic influenza seed virus. The pharma did not disclose the status of its application.

Weakly immunogenic antigens are another source of inefficiency in
(Continues on p. 7)

Optimizing transferrin-mediated transcytosis

By Kai-Jye Lou, Senior Writer

Researchers at the **California Institute of Technology** have shown how modulating the transferrin content on gold nanoparticles can optimize their delivery into the brain via the transferrin receptor.¹ The group now needs to determine whether its strategy will translate to drug-nanoparticle conjugates amenable for use in the CNS.

The normal function of the transferrin receptor is to engage with transferrin to bring the iron-binding protein into cells that express the receptor. In the endothelial cells that line the vasculature of the brain, the receptor helps shuttle transferrin from one side of the blood brain barrier (BBB) to the other—a process called receptor-mediated transcytosis.²

Prior efforts to exploit the transferrin receptor's mechanism to improve the delivery of therapeutic agents into the brain involved conjugating the agents to transferrin receptor-binding mAbs or transferrin-conjugated nanoparticles.³⁻⁵ However, few such conjugates were successful.

A key breakthrough came in 2011 when researchers at **Roche's Genentech Inc.** unit realized that antibodies with high affinity for the transferrin receptor were being trapped in the endothelium because they remained bound to the receptor.⁶⁻⁸

This led the Genentech group to develop lower-affinity mAbs that would be released from the receptor after crossing the BBB and accumulate in the brain at higher concentrations than high-affinity mAbs.

“We want to use the data generated in our study as a basis for understanding how to design other classes of nanoparticles that could be used in the therapeutic setting to target the brain, such as polymeric or liposomal nanoparticles.”

—Devin Wiley,
Keck School of Medicine of the
University of Southern California

The Caltech group has now found that a similar principle applies to transferrin-conjugated nanoparticles.

The researchers synthesized a series of gold nanoparticles of different diameters and surface transferrin content. In mice, nanoparticles with diameters of 40–80 nm and moderate transferrin content bound to the transferrin receptor, penetrated the BBB and accumulated in the brain parenchyma.

In contrast, nanoparticles with low transferrin content showed weak binding to the transferrin receptor and failed to penetrate the BBB. Those with high transferrin content bound to the receptor but appeared to stick on or in endothelial cells (*see Figure 1, “Model of receptor-mediated transcytosis of transferrin-containing nanoparticles”*).

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

The researchers used gold nanoparticles in the model system because they are easy to modify and visualize. Similar gold nanoparticles are in clinical trials for non-CNS conditions, but they are not biodegradable and are unlikely to be suitable for clinical use in the CNS.

“We carried out this study because we wanted to translate the idea of modulating transferrin receptor binding in antibodies to optimizing a nanoparticle's ability to cross the BBB and access the brain,” said Devin Wiley, lead author on the paper, who is now a medical student at the **Keck School of Medicine of the University of Southern California**. “We want

to use the data generated in our study as a basis for understanding how to design other classes of nanoparticles that could be used in the therapeutic setting to target the brain, such as polymeric or liposomal nanoparticles.”

“The data in this study, at a general level, suggest that the density of
(Continues on p. 8)

(Continued from “**Synthetic influenza seeds**,” p. 6)

the production of vaccine. As a result, large quantities of the antigens are needed for efficacy. According to CIDRAP, most vaccines in clinical trials use the same immunologic approach as current vaccines.

Thus, to dramatically shorten the time it takes to get a vaccine to the public in response to an influenza pandemic, the immunogenicity of antigens needs to be improved.

IP for different portions of the synthetic processes and the cell lines described in the *Science Translational Medicine* report are owned by Novartis, Synthetic Genomics and **MedImmune LLC**.

Donner, A. *SciBX* 6(21); doi:10.1038/scibx.2013.509
published online May 30, 2013

REFERENCES

- Dormitzer, P.R. *et al. Sci. Transl. Med.*; published online May 15, 2013; doi:10.1126/scitranslmed.3006368
Contact: Philip R. Dormitzer, Novartis Vaccines and Diagnostics, Cambridge, Mass.
e-mail: philip.dormitzer@novartis.com

- Gibson, D.G. *et al. Nat. Methods* 7, 901–903 (2010)
- Hoffmann, E. *et al. Proc. Natl. Acad. Sci. USA* 97, 6108–6113 (2000)
- Osterholm, M.T. *et al. The compelling need for game-changing influenza vaccines*. (CIDRAP, Oct. 15, 2012)

COMPANIES AND INSTITUTIONS MENTIONED

Biomedical Advanced Research and Development Authority, Washington, D.C.
Center for Infectious Disease Research & Policy, Minneapolis, Minn.
Codagenix Inc., Stony Brook, N.Y.
J. Craig Venter Institute, Rockville, Md.
MedImmune LLC, Gaithersburg, Md.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Philipps University of Marburg, Marburg, Germany
Synthetic Genomics Inc., La Jolla, Calif.
University of Minnesota, Minneapolis, Minn.
World Health Organization, Geneva, Switzerland

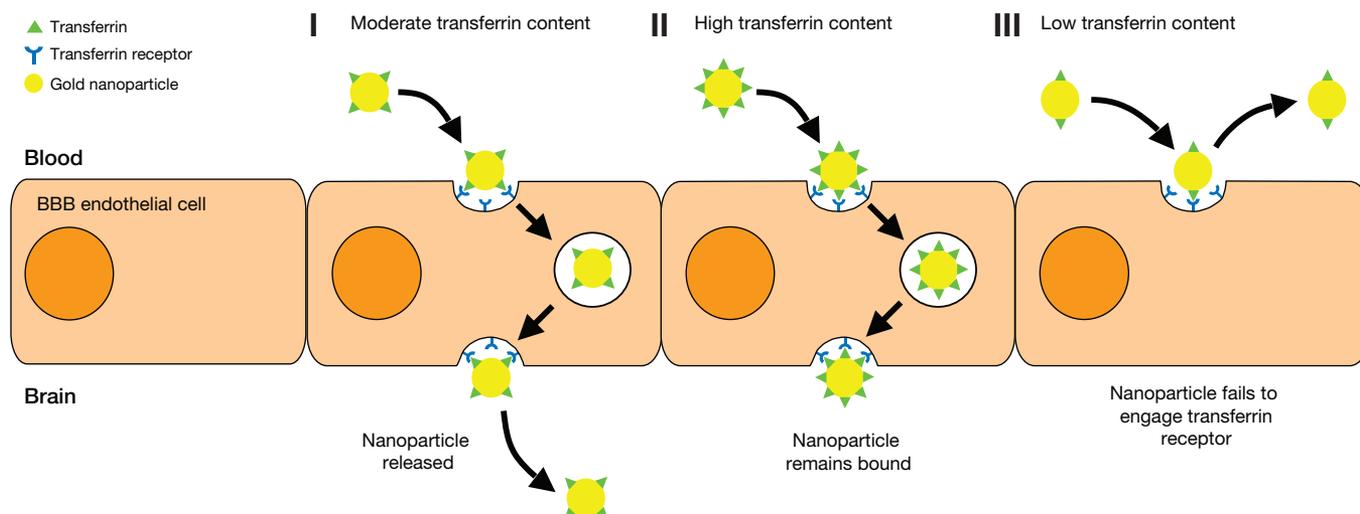


Figure 1. Model of receptor-mediated transcytosis of transferrin-containing nanoparticles. Transferrin and its receptor are one of the ligand-receptor combinations that could be exploited to transport molecules across the blood brain barrier (BBB). As reported in Wiley *et al.*, researchers found that gold nanoparticles with moderate transferrin content were most effective at crossing the BBB into the brain parenchyma. The researchers proposed a mechanism underlying their observations. Nanoparticles with moderate transferrin content (I) engage the transferrin receptor on BBB endothelial cells, undergo receptor-mediated transcytosis to traverse the BBB and are then released into the brain parenchyma. Nanoparticles with high transferrin content (II) remain associated with BBB endothelial cells and fail to be released into the brain parenchyma after receptor-mediated transcytosis. On the other hand, nanoparticles with low transferrin content (III) fail to engage the transferrin receptor on BBB endothelial cells.

transferrin molecules on a nanoparticle can make a difference in how well it penetrates the BBB,” said Pieter Gaillard, cofounder and CSO of **to-BBB technologies B.V.**

“This study confirms the results of earlier studies carried out by our group and others showing that transferrin-coated nanoparticles can help enhance the delivery of drugs into the brain,” added Jörg Kreuter, a professor at the Institute for Pharmaceutical Technology at **Goethe University Frankfurt**.

In 2009, Kreuter’s group reported that i.v. infusion of human serum albumin nanoparticles loaded with loperamide and covalently coupled to transferrin or transferrin receptor-binding antibodies induced antinociceptive effects in mice.⁹ Loperamide is a generic opioid that does not cross the BBB under normal circumstances.

Both Gaillard and Kreuter cautioned against generalizing these results with the gold nanoparticle system to other nanoparticles.

Kreuter said that his own group’s work has shown that conjugating the same targeting ligand to different types of nanoparticles can yield molecules that behave very differently from one another. He added that the agent to be delivered by the nanoparticle could itself significantly alter the nanoparticle’s properties.

to-BBB uses liposomes coated with glutathione-conjugated polyethylene glycol (PEG) to improve drug delivery across the BBB. The company’s 2B3-101, a liposomal formulation of doxorubicin coated with glutathione-conjugated PEG, is in Phase I/IIa testing to treat patients who have malignant glioma or solid tumors with metastasis to the brain.

The company plans to start the study’s Phase IIa portion this year. Genentech declined to comment.

Moving toward clinical relevance

Kreuter thinks an important next step will be identifying a relevant drug-nanoparticle combination and disease in which to test the transferrin-based approach.

Gaillard said that the researchers will need to do additional pharmacological legwork to confirm that their transferrin-coated nanoparticles are indeed crossing the BBB via receptor-mediated transcytosis and that the reported differences in reaching the brain parenchyma are due to variations in the nanoparticles’ ability to bind the transferrin receptor.

“The researchers still need to show that the nanoparticles are not getting into the brain via some other mechanism or are the result of an artifact of the analytical method or experimental setup,” he told *SciBX*.

Gaillard said that the Caltech researchers also must assess how the plasma and brain concentrations of transferrin-coated nanoparticles vary across multiple time points using pharmacologically relevant dosing regimens or at steady-state plasma levels. He also thinks that future studies should use nanoparticles that are more amenable than gold particles for drug delivery to the brain.

These would include nanoparticles created from biodegradable materials, such as poly(alkyl cyanoacrylates), poly(lactic-co-glycolic acid), albumin or chitosan, according to Kreuter.

Wiley said that the Caltech group is adapting the transferrin-based approach to polymeric nanoparticle systems that deliver therapeutic agents. The researchers are also trying to identify additional parameters that would be relevant to a nanoparticle’s ability to cross the BBB and to understand how to modulate such parameters.

Wiley said that the potential therapeutic settings being considered

are diseases of the brain where the BBB is known to exclude existing therapeutic agents, such as Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD) and brain cancer.

Caltech has filed for a patent covering methods to deliver nanoparticles to the brain. The technology is not available for licensing.

Lou, K.-J. *SciBX* **6**(21); doi:10.1038/scibx.2013.510

Published online May 30, 2013

REFERENCES

1. Wiley, D.T. *et al. Proc. Natl. Acad. Sci. USA*; published online May 6, 2013; doi:10.1073/pnas.1307152110
Contact: Mark E. Davis, California Institute of Technology, Pasadena, Calif.
e-mail: mdavis@cheme.caltech.edu
2. Descamps, L. *et al. Am. J. Physiol.* **270**, H1149–H1158 (1996)

3. Bickel, U. *et al. Adv. Drug Deliv. Rev.* **46**, 247–279 (2001)
4. Chang, J. *et al. Int. J. Pharm.* **379**, 285–292 (2009)
5. Karatas, H. *et al. J. Neurosci.* **29**, 13761–13769 (2009)
6. Osherovich, L. *SciBX* **4**(22); doi:10.1038/scibx.2011.619
7. Atwal, J.K. *et al. Sci. Transl. Med.* **3**, 84ra43 (2011)
8. Yu, Y.J. *et al. Sci. Transl. Med.* **3**, 84ra44 (2011)
9. Ulbrich, K. *et al. Eur. J. Pharm. Biopharm.* **71**, 251–256 (2009)

COMPANIES AND INSTITUTIONS MENTIONED

California Institute of Technology, Pasadena, Calif.

Goethe University Frankfurt, Frankfurt, Germany

Genentech Inc., South San Francisco, Calif.

Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland

to-BBB technologies B.V., Leiden, the Netherlands

Keck School of Medicine of the University of Southern

California, Los Angeles, Calif.

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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				
Brain cancer	Aldehyde dehydrogenase 1 family member A3 (ALDH1A3)	<p>Patient sample and mouse studies suggest inhibiting ALDH1A3 could help treat a subset of high-grade gliomas. In glioma stem cell cultures derived from 40 samples from patients with high-grade glioma, analysis of gene expression signatures identified two distinct subsets of glioma stem cells—mesenchymal and proneural. In intracranial mouse xenograft models, mesenchymal glioma stem cells were resistant to radiation therapy and showed greater proliferation than proneural glioma stem cells. In the glioma stem cells, small hairpin RNA-mediated <i>ALDH1A3</i> knockdown inhibited growth of mesenchymal but not proneural glioma stem cells. Next steps include developing inhibitors of ALDH1A3.</p> <p>SciBX 6(21); doi:10.1038/scibx.2013.511 Published online May 30, 2013</p>	Patent application filed; available for licensing	<p>Mao, P. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online May 6, 2013; doi:10.1073/pnas.1221478110 Contact: Ichiro Nakano, The Ohio State University, Columbus, Ohio e-mail: ichiro.nakano@osumc.edu Contact: Robert W. Sobol, University of Pittsburgh School of Medicine, Pittsburgh, Pa. e-mail: rws9@pitt.edu Contact: Shi-Yuan Cheng, Northwestern University Feinberg School of Medicine, Chicago, Ill. e-mail: shiyuan.cheng@northwestern.edu</p>
Cancer	Baculoviral IAP repeat-containing 2 (BIRC2; cIAP1); cIAP2 (BIRC3); diablo homolog (DIABLO; SMAC); X-linked inhibitor of apoptosis (XIAP)	<p>Rodent and <i>in vitro</i> studies identified an orally bioavailable, bivalent SMAC mimetic that could help treat cancer. <i>In vitro</i>, the bivalent SMAC mimetic SM-1200 bound to cIAP1, cIAP2 and XIAP and inhibited growth of human breast and ovarian cancer cell lines with nanomolar IC₅₀ values. In rats, SM-1200 showed greater oral bioavailability than related bivalent mimetics. In a mouse xenograft model for human breast cancer, SM-1200 caused greater tumor regression than docetaxel. Next steps include <i>in vivo</i> studies to evaluate SM-1200 and related analogs as monotherapy or in combination with other drugs in cancer models. Ascentage Pharma Group Corp. Ltd. and 3SBio Inc. have a multi-cIAP inhibitor in preclinical development to treat solid tumors. At least seven companies have SMAC mimetics in Phase II testing or earlier to treat various cancers.</p> <p>SciBX 6(21); doi:10.1038/scibx.2013.512 Published online May 30, 2013</p>	Patented by the University of Michigan; licensed to Ascentage Pharma Group	<p>Sheng, R. <i>et al. J. Med. Chem.</i>; published online May 7, 2013; doi:10.1021/jm400216d Contact: Shaomeng Wang, University of Michigan, Ann Arbor, Mich. e-mail: shaomeng@umich.edu</p>
Cancer	Lysine-specific demethylase 4A (KDM4A; JMJD2A); jun (AP1) proto-oncogene	<p>Cell culture and mouse studies suggest inhibiting KDM4A could help treat metastatic squamous cell carcinoma (SCC). A small hairpin RNA screen against 27 histone demethylases (HDACs) in SCC cells showed that <i>KDM4A</i> knockdown decreased cellular invasion and expression of AP1 target genes compared with no knockdown. In an orthotopic mouse model for SCC, shRNA against <i>KDM4A</i> in implanted cells decreased metastasis compared with control shRNA. Next steps include testing KDM4A inhibitors in SCC models.</p> <p>SciBX 6(21); doi:10.1038/scibx.2013.513 Published online May 30, 2013</p>	Unpatented; licensing status not applicable	<p>Ding, X. <i>et al. Sci. Signal.</i>; published online April 30, 2013; doi:10.1126/scisignal.2003884 Contact: Cun-Yu Wang, University of California, Los Angeles, Calif. e-mail: cunywang@ucla.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer	Macrophage stimulating 1 receptor c-Met-related tyrosine kinase (MST1R; RON; CD136); c-Met proto-oncogene (MET; HGFR)	<p>Mouse studies suggest inhibiting RON could help treat metastatic cancers by inducing an antitumor immune response. In an immune-competent mouse model for metastatic breast cancer, knockout of <i>Mst1r</i> eliminated metastasis and induced a more robust CD8⁺ T cell antitumor response than no knockout. ASLAN002, an inhibitor of MET and RON, decreased metastasis to the lungs of mice compared with vehicle. Next steps include combination studies with chemotherapy.</p> <p>Aslan Pharmaceuticals Pte. Ltd.'s ASLAN002 (formerly BMS-777607) is in Phase I trials to treat cancer.</p> <p>Eli Lilly and Co.'s narnatumab (IMC-RON8), an anti-RON mAb, is in Phase I testing to treat cancer (<i>see RON's new role, page 1</i>).</p> <p>SciBX 6(21); doi:10.1038/scibx.2013.514 Published online May 30, 2013</p>	Unpatented; licensing status not applicable	<p>Eyob, H. <i>et al. Cancer Discov.</i>; published online April 23, 2013; doi:10.1158/2159-8290.CD-12-0480</p> <p>Contact: Alana L. Welm, Huntsman Cancer Institute at The University of Utah, Salt Lake City, Utah</p> <p>e-mail: alana.welm@hci.utah.edu</p>
Cancer	Mdm2 p53 binding protein homolog (MDM2; HDM2); p53	<p><i>In vitro</i> and mouse studies identified MI-888 as an inhibitor of MDM2-p53 protein-protein interactions that could help treat cancer. In the <i>in vitro</i> binding studies, MI-888 showed subnanomolar binding affinity for MDM2. In a mouse xenograft model for human osteosarcoma, MI-888 caused complete tumor regression, whereas vehicle did not. Mice remained tumor free for at least 64 days after the last dose of MI-888. Next steps include testing and optimizing MI-888 for use in multiple tumor types.</p> <p>Ascenta Therapeutics Inc. and Sanofi have MI-773, a related small molecule inhibitor of p53-MDM2 protein-protein interactions, in Phase I testing to treat cancer.</p> <p>SciBX 6(21); doi:10.1038/scibx.2013.515 Published online May 30, 2013</p>	Patent applications filed; licensed to Ascenta and sublicensed to Sanofi	<p>Zhao, Y. <i>et al. J. Am. Chem. Soc.</i>; published online May 3, 2013; doi:10.1021/ja3125417</p> <p>Contact: Shaomeng Wang, University of Michigan, Ann Arbor, Mich.</p> <p>e-mail: shaomeng@umich.edu</p>
Cancer	Signal transducer and activator of transcription 3 (STAT3)	<p><i>In vitro</i> and mouse studies suggest a new STAT3 inhibitor could help treat cancer. Fragment-based drug design was used to create a series of STAT3 inhibitors. In a panel of human cancer cell lines, a lead naphthalene-sulfonamide analog inhibited STAT3 phosphorylation with nanomolar to low micromolar IC₅₀ values. In a mouse xenograft model for human breast cancer, the lead compound decreased tumor growth compared with vehicle. Ongoing work includes testing the lead compound in mouse models for sarcoma and pancreatic cancer.</p> <p>Isis Pharmaceuticals Inc. and AstraZeneca plc have ISIS-STAT3Rx, an antisense inhibitor of STAT3, in Phase II testing to treat cancer.</p> <p>Otsuka Pharmaceutical Co. Ltd.'s OPB-31121, an inhibitor of STAT3 phosphorylation, is in Phase I testing to treat cancer.</p> <p>GLG Pharma LLC has at least four inhibitors of activated STAT3 or STAT3 phosphorylation in preclinical testing to treat cancer.</p> <p>SciBX 6(21); doi:10.1038/scibx.2013.516 Published online May 30, 2013</p>	Patent application filed by The Ohio State University; available for licensing	<p>Yu, W. <i>et al. J. Med. Chem.</i>; published online May 7, 2013; doi:10.1021/jm400080c</p> <p>Contact: Chenglong Li, The Ohio State University, Columbus, Ohio</p> <p>e-mail: li.728@osu.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Ovarian cancer	Nicotinamide phosphoribosyl transferase (NAMPT; NamPRT)	<i>In vitro</i> and mouse studies identified thiourea inhibitors of NAMPT that could be useful for treating ovarian cancer. In cultured ovarian tumor cells, SAR studies identified a lead thiourea-based compound that inhibited NAMPT with an IC ₅₀ value of 32 nM. In mouse xenograft models for human ovarian cancer, the most potent compounds decreased tumor growth compared with vehicle and showed oral bioavailability. Next steps include further chemical optimization.	Patent and licensing status undisclosed	Zheng, X. <i>et al. J. Med. Chem.</i> ; published online April 25, 2013; doi:10.1021/jm400186h Contact: Xiaozhang Zheng, Forma Therapeutics Inc., Watertown, Mass. e-mail: xzheng@formatherapeutics.com
Cardiovascular disease				
Thrombosis	Peptidyl arginine deiminase type IV (PAD4; PAD4)	Mouse studies suggest PAD4 inhibition could help treat or prevent thrombosis. PAD4 is required for the formation of neutrophil extracellular traps, which are components of thrombi. In a mouse model for deep vein thrombosis, thrombi formed in less than 10% of <i>Padi4</i> -deficient animals, whereas thrombi formed in more than 90% of wild-type animals. <i>Padi4</i> knockout did not affect normal bleeding and platelet function. Next steps include developing inhibitors of PAD4.	Patent application filed; available for licensing	Martinod, K. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online May 6, 2013; doi:10.1073/pnas.1301059110 Contact: Denisa D. Wagner, Harvard Medical School, Boston, Mass. e-mail: denisa.wagner@childrens.harvard.edu
Endocrine/metabolic disease				
Diabetes; obesity	Not applicable	Mouse studies suggest molecules produced by the bacterial strain <i>Akkermansia muciniphila</i> could help treat obesity and type 2 diabetes. In a mouse model for obesity, oral delivery of <i>A. muciniphila</i> normalized metabolic markers of adiposity and reversed diet-induced fasting hyperglycemia, and it decreased body weight compared with no treatment. In mouse models for diabetes and obesity, treatment with oligofructose to increase <i>A. muciniphila</i> abundance led to decreased total fat mass and body weight compared with no treatment. Next steps include identifying the bacterial molecules that mediate the observed effects and evaluating their therapeutic potential.	Unpatented; licensing status undisclosed	Everard, A. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online May 13, 2013; doi:10.1073/pnas.1219451110 Contact: Patrice D. Cani, Université Catholique de Louvain, Brussels, Belgium e-mail: patrice.cani@uclouvain.be
Infectious disease				
Staphylococcus	Accessory gene regulator cognate receptor (AgrC receptor)	An <i>in vitro</i> study suggests autoinducing peptide III (AIP-III) mimetics that inhibit AgrC receptors could help treat toxic shock syndrome and <i>Staphylococcus aureus</i> infections. In cultured <i>S. aureus</i> cells, analysis of AIP-III-based macrocyclic peptides led to the development of peptide mimetics that inhibit AgrC receptors with picomolar potency. In coculture, addition of the peptide mimetics decreased <i>S. aureus</i> hemolysis compared with addition of native AIP peptides. <i>In vitro</i> , the peptide mimetics decreased toxic shock syndrome toxin-1 production by <i>S. aureus</i> compared with control peptides. Next steps include identifying the mechanisms of action for the analogs, increasing their potency and stability and testing them in animal models for infection.	Patent application filed; available for licensing from the Wisconsin Alumni Research Foundation at the University of Wisconsin–Madison	Tal-Gan, Y. <i>et al. J. Am. Chem. Soc.</i> ; published online May 6, 2013; doi:10.1021/ja3112115 Contact: Helen E. Blackwell, University of Wisconsin–Madison, Madison, Wis. e-mail: blackwell@chem.wisc.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Musculoskeletal disease				
Bone repair	Myocilin (MYOC)	Cell culture and mouse studies suggest MYOC could improve the efficacy of mesenchymal stem cell (MSC) therapy for bone repair. In both mouse and human MSCs, addition of MYOC increased differentiation into osteoblasts compared with no MYOC addition. <i>Myoc</i> -deficient mice showed decreased cortical bone thickness compared with wild-type mice. Next steps could include identifying the molecular target by which MYOC enhances MSC differentiation into osteoblasts and developing molecules against the target. SciBX 6(21); doi:10.1038/scibx.2013.521 Published online May 30, 2013	Unpatented; licensing status not applicable	Kwon, H.S. <i>et al. J. Biol. Chem.</i> ; published online April 29, 2013; doi:10.1074/jbc.M112.422972 Contact: Stanislav I. Tomarev, National Institutes of Health, Bethesda, Md. e-mail: tomarevs@nei.nih.gov
Neurology				
Alzheimer's disease (AD)	Legumain (LGMN; AEP)	Patient sample, tissue culture and <i>in vitro</i> studies suggest inhibiting AEP could help treat AD. In homogenates derived from patient frontal cortex samples, AEP activity was greater than that in samples from healthy controls. In rat hippocampal slices, acidosis activated AEP and led to microtubule-associated protein- τ (MAPT; TAU; FTDP-17) hyperphosphorylation, a hallmark of AD. In a human neuronal cell line, small interfering RNA-mediated AEP knockdown resulted in decreased hyperphosphorylated TAU levels compared with no knockdown. Next steps include evaluating brain-specific knockdown of AEP in mouse models for AD and screening for small molecules that inhibit the protein. SciBX 6(21); doi:10.1038/scibx.2013.522 Published online May 30, 2013	Unpatented; licensing status not applicable	Basurto-Islas, G. <i>et al. J. Biol. Chem.</i> ; published online May 2, 2013; doi:10.1074/jbc.M112.446070 Contact: Khalid Iqbal, The Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y. e-mail: khalid.iqbal.ibr@gmail.com
Depression	Not applicable	Mouse studies suggest increasing ATP release from astrocytes could help treat depression. In mouse models for depression, intracerebroventricular infusion of ATP decreased depressive behaviors more rapidly than the generic antidepressant imipramine. In normal mice, inhibition of ATP release from astrocytes induced depressive behaviors. In mouse models for depression, intracerebroventricular infusion of ATP decreased depressive behaviors compared with vehicle. Next steps could include screening for compounds that stimulate ATP release from astrocytes. SciBX 6(21); doi:10.1038/scibx.2013.523 Published online May 30, 2013	Patent and licensing status unavailable	Cao, X. <i>et al. Nat. Med.</i> ; published online May 5, 2013; doi:10.1038/nm.3162 Contact: Tian-Ming Gao, Southern Medical University, Guangzhou, China e-mail: tgao@smu.edu.cn Contact: Xin-Hong Zhu, same affiliation as above e-mail: zhuxh@smu.edu.cn
Depression	Serum/ glucocorticoid regulated kinase 1 (SGK1)	Patient, rodent and cell culture studies suggest inhibiting SGK1 could help treat stress-induced depression. In a human hippocampal progenitor cell line, a small molecule inhibitor of SGK1 blocked cortisol-induced decreases in neurogenesis, whereas vehicle did not. In peripheral blood samples from patients with major depression, SGK1 mRNA levels were higher than those in healthy controls. In two rat models of stress-induced depression, hippocampal expression of Sgk1 was greater than that in nonstressed controls. Next steps include determining whether inhibition of SGK1 can block the effects of stress and stress hormones on brain function and depression-like behaviors in animal models. SciBX 6(21); doi:10.1038/scibx.2013.524 Published online May 30, 2013	Unpatented; licensing status not applicable	Anacker, C. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online May 6, 2013; doi:10.1073/pnas.1300886110 Contact: Christoph Anacker, King's College London, London, U.K. e-mail: christoph.anacker@kcl.ac.uk

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Neurology	Sirtuin 1 (SIRT1)	<i>In vitro</i> and mouse studies suggest inhibiting SIRT1 could help treat myelination disorders. In a mouse model for lysolecithin-induced demyelination, genetic inactivation of <i>Sirt1</i> increased remyelination compared with <i>Sirt1</i> activation. In a mouse model for experimental autoimmune encephalomyelitis (EAE), <i>Sirt1</i> inactivation delayed paralysis. Next steps include testing pharmacological inhibitors of SIRT1 in animals. Elixir Pharmaceuticals Inc. and Siena Biotech S.p.A. have the SIRT1 inhibitor EX-527 in Phase I testing to treat Huntington's disease (HD).	Findings unpatented; licensing status not applicable	Rafalski, V.A. <i>et al. Nat. Cell Biol.</i> ; published online May 5, 2013; doi:10.1038/ncb2735 Contact: Anne Brunet, Stanford University School of Medicine, Stanford, Calif. e-mail: anne.brunet@stanford.edu
		SciBX 6(21); doi:10.1038/scibx.2013.525 Published online May 30, 2013		
Parkinson's disease (PD)	Adenosine A _{2A} receptor (ADORA _{2A}); monoamine oxidase B (MAO-B)	<i>In vitro</i> studies suggest dual antagonists of ADORA _{2A} and MAO-B could be useful for treating PD. Both proteins are known targets in PD. <i>In vitro</i> , the lead molecule from a series of benzothiazinones selectively antagonized human ADORA _{2A} with an IC ₅₀ value of 39.5 nM and MAO-B with an IC ₅₀ value of 34.9 nM. Next steps include evaluating other members of the compound series in mice. Kyowa Hakko Kirin Co. Ltd. markets the ADORA _{2A} antagonist Nouriasit istradefylline to treat PD. At least seven other companies have ADORA _{2A} antagonists in Phase III or earlier testing to treat PD. Teva Pharmaceutical Industries Ltd. and H. Lundbeck A/S market Azilect rasagiline, an irreversible selective inhibitor of MAO-B, to treat PD. Valeant Pharmaceuticals International Inc. markets the MAO-B inhibitor Zelapar selegiline for the same indication. At least six other MAO-B inhibitors are in Phase III or earlier testing to treat PD.	Compound series covered by pending patents; available for licensing	Stöbel, A. <i>et al. J. Med. Chem.</i> ; published online April 30, 2013; doi:10.1021/jm400336x Contact: Christa E. Müller, PharmaCenter Bonn, Bonn, Germany e-mail: christa.mueller@uni-bonn.de Contact: Michael Gütschow, same affiliation as above e-mail: guetschow@uni-bonn.de
		SciBX 6(21); doi:10.1038/scibx.2013.526 Published online May 30, 2013		
Ophthalmic disease				
Age-related macular degeneration (AMD); retinitis	PC4 and SFRS1-interacting protein (PSIP1; LEDGF; p75; LEDGF/p75)	Cell culture and rat studies suggest the lens epithelium-derived growth factor fragment LEDGF ₁₋₃₂₆ could help treat dry AMD and retinitis pigmentosa (RP). In retinal cell culture, the fragment decreased protein aggregation-mediated stress and cell death compared with no protein fragment. In a rat model for dry AMD and RP, intravitreal injection of LEDGF ₁₋₃₂₆ decreased photoreceptor loss compared with vehicle injection. Next steps include evaluating the safety of LEDGF ₁₋₃₂₆ compositions in animal models and assessing the effectiveness of using the protein fragment to treat protein aggregation diseases of the brain.	Patent application filed covering compositions and methods of treatment; available for licensing from the University of Colorado Denver Technology Transfer Office Contact: David Poticha, University of Colorado Denver, Aurora, Colo. e-mail: david.poticha@cu.edu	Baid, R. <i>et al. J. Biol. Chem.</i> ; published online May 2, 2013; doi:10.1074/jbc.M112.441618 Contact: Uday B. Kompella, University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences, Aurora, Colo. e-mail: uday.kompella@ucdenver.edu
		SciBX 6(21); doi:10.1038/scibx.2013.527 Published online May 30, 2013		

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			
Small molecule fruit fly screening platform for regulators of sleep	A small molecule fruit fly screening platform could be useful for identifying new candidates to treat insomnia. Adult fruit flies are placed in tubes with food containing 20 μ M of a test compound and then loaded into an activity monitoring system. The screening system was applied to a library of 1,280 bioactive small molecules and identified reserpine, a small molecule inhibitor of vesicular monoamine transporters (VMATs), as a compound that increased sleep in the flies. Follow-up validation studies showed that <i>Vmat</i> -null flies slept longer than wild-type controls. Next steps include using the screen to study how different neurotransmitter systems affect sleep behavior. Reserpine is a generic antipsychotic and antihypertensive drug. SciBX 6(21); doi:10.1038/scibx.2013.528 Published online May 30, 2013	Unpatented; licensing status not applicable	Nall, A.H. & Sehgal, A. <i>J. Neurosci.</i> ; published online May 8, 2013; doi:10.1523/JNEUROSCI.0253-13.2013 Contact: Amita Sehgal, University of Pennsylvania School of Medicine, Philadelphia, Pa. e-mail: amita@mail.med.upenn.edu
Whole-exome sequencing to identify antigens for adoptively transferred, autologous tumor-infiltrating lymphocytes (TILs)	Whole-exome sequencing could help to identify mutated antigens to express in TILs for adoptive transfer immunotherapy. Whole-exome sequencing identified nonsilent, somatic mutations in the DNA of melanoma cells that were absent from that of matched normal cells. A peptide-major histocompatibility complex (MHC) binding algorithm was then used to select high-affinity candidate T cell epitopes that would be recognized by TILs. Top candidate epitopes were synthesized and screened for their ability to induce interferon- γ (IFN γ) release from TIL cell lines derived from three patients with melanoma. Next steps include evaluating the association between TIL recognition of mutant antigens and long-term tumor regression. SciBX 6(21); doi:10.1038/scibx.2013.529 Published online May 30, 2013	Unpatented; licensing status not applicable	Robbins, P.F. <i>et al. Nat. Med.</i> ; published online May 5, 2013; doi:10.1038/nm.3161 Contact: Paul F. Robbins, National Institutes of Health, Bethesda, Md. e-mail: paulrobbins@mail.nih.gov
Disease models			
Human embryonic stem cells (hESCs) derived from somatic cell nuclear transfer (SCNT)	SCNT could be useful for creating patient-matched hESCs for disease modeling and therapeutic applications. Previous efforts to use nuclear transfer to generate hESCs have not been successful because the cells undergo early embryonic arrest after nuclear transfer and do not yield stable cell lines. In donated human oocytes, an optimized SCNT protocol was used to fuse a fibroblast derived from a human cell line with an enucleated donor human oocyte. A subset of the resulting oocytes developed into blastocysts, which were used to establish stable hESC lines bearing the genome of the donor fibroblast. Next steps could include comparing the hESC lines generated with other pluripotent stem cell lines. SciBX 6(21); doi:10.1038/scibx.2013.530 Published online May 30, 2013	Patents pending; available for licensing	Tachibana, M. <i>et al. Cell</i> ; published online May 15, 2013; doi:10.1016/j.cell.2013.05.006 Contact: Shoukhrat Mitalipov, Oregon Health & Science University, Portland, Ore. e-mail: mitalipo@ohsu.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Drug delivery			
Transferrin-coated, brain-penetrant gold nanoparticles	<p>Mouse studies suggest transferrin-coated gold nanoparticles could be useful for delivering therapeutics across the blood brain barrier (BBB). Gold nanoparticles of different diameters and transferrin content were synthesized. In mice, nanoparticles with diameters of 40 nm or 80 nm and moderate transferrin content bound to the transferrin receptor, penetrated the BBB and accumulated in the brain parenchyma. In contrast, gold nanoparticles with low transferrin content showed weak binding to the transferrin receptor and failed to penetrate the BBB, whereas those with high transferrin content bound to the receptor but were not released into the brain parenchyma. Next steps include adapting the transferrin-based strategy to polymeric nanoparticle technologies and exploring other nanoparticle parameters that could be modulated to optimize delivery across the BBB (see <i>Optimizing transferrin-mediated transcytosis</i>, page 7).</p> <p>SciBX 6(21); doi:10.1038/scibx.2013.531 Published online May 30, 2013</p>	Patent application filed covering methods to deliver nanoparticles to the brain; unavailable for licensing	<p>Wiley, D.T. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online May 6, 2013; doi:10.1073/pnas.1307152110</p> <p>Contact: Mark E. Davis, California Institute of Technology, Pasadena, Calif. e-mail: mdavis@cheme.caltech.edu</p>
Drug platforms			
Efficient generation of seed virus for production of synthetic influenza vaccines	<p>A synthetic approach for assembling influenza A virus genome segments could improve the efficiency of generating seed virus for vaccine production. A mostly automated, one-step, oligonucleotide-based assembly process with error correction increased the accuracy in genome segment assembly from about 3% to more than 80%. In cells approved for vaccine manufacture, seed virus was recovered from cells transfected with the genome segments and viral backbone elements. In a simulated pandemic setting, the synthetic method yielded seed virus in 4 days and 4 hours, whereas methods used in response to the 2009 influenza pandemic required 36 days. Next steps include automating additional steps of the seed virus-generation process and applying this method to generate RNA vaccines (see <i>Synthetic influenza seeds</i>, page 6).</p> <p>SciBX 6(21); doi:10.1038/scibx.2013.532 Published online May 30, 2013</p>	Patented by Novartis AG, Synthetic Genomics Inc. and MedImmune LLC; available for licensing	<p>Dormitzer, P.R. <i>et al. Sci. Transl. Med.</i>; published online May 15, 2013; doi:10.1126/scitranslmed.3006368</p> <p>Contact: Philip R. Dormitzer, Novartis Vaccines and Diagnostics, Cambridge, Mass. e-mail: philip.dormitzer@novartis.com</p>
IL-15 and soluble IL-15 receptor α -chain (sIL-15RA) heterodimers	<p>Mouse studies suggest heterodimers of IL-15 and sIL-15RA could be more potent than IL-15 monomers for stimulating the immune system against diseases such as cancer. Cell lines that produce IL-15 and sIL-15RA were used to generate stable heterodimers of the molecules. In mice, the heterodimers showed longer half-life, higher plasma stability and more potent stimulation of lymphocyte proliferation than did IL-15 monomers. Next steps include scaling up the process to generate GMP material as well as a planned Phase I trial in cancer.</p> <p>SciBX 6(21); doi:10.1038/scibx.2013.533 Published online May 30, 2013</p>	Patent application filed; licensed to Marine Polymer Technologies Inc.	<p>Chertova, E. <i>et al. J. Biol. Chem.</i>; published online May 6, 2013; doi:10.1074/jbc.M113.461756</p> <p>Contact: George N. Pavlakis, National Cancer Institute, Bethesda, Md. e-mail: pavlakig@mail.nih.gov</p>
Markers			
Genetic markers underlying respiratory droplet transmission of influenza virus	<p>Reverse genetics and guinea pig studies identified components of influenza virus that enable respiratory droplet transmission, which could aid epidemiological studies. The H1N1 influenza strain A/Sichuan/1/2009 is transmissible via respiratory droplets, whereas the H5N1 strain A/duck/Guangxi/35/2001 is not. Plasmid-based reverse genetics was used to generate 127 hybrids of the 2 influenza strains that retained H5 hemagglutinin. In guinea pigs, hybrids bearing the acidic polymerase and nonstructural protein gene of the H1N1 strain were shown to be transmissible via respiratory droplets. Next steps could include studies to determine how the H1N1 acidic polymerase and nonstructural protein enable respiratory droplet transmission of the influenza virus.</p> <p>SciBX 6(21); doi:10.1038/scibx.2013.534 Published online May 30, 2013</p>	Patent and licensing status unavailable	<p>Zhang, Y. <i>et al. Science</i>; published online May 2, 2013; doi:10.1126/science.1229455</p> <p>Contact: Hualan Chen, Chinese Academy of Agricultural Sciences, Harbin, China e-mail: hlchen1@yahoo.com</p>

Erratum: Analysis: Cover StoryOsherovich, L. *SciBX* 6(20); doi:10.1038/scibx.2013.478

Published online May 23, 2013

The Analysis item "IRS audit for tumors" omitted one of Alexander Levitzki's titles and misstated the founders of NovoTyr Therapeutics Ltd. Levitzki is CSO of NovoTyr and was the sole founder of the company.

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