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Bringing on the BRAF

By Tracey Baas, Senior Editor

The BRAF inhibitor Zelboraf vemurafenib from **Daiichi Sankyo Co. Ltd.** and **Roche** produces about an 80% response rate in its approved indication of BRAF-mutant melanoma but only about a 5% rate in colorectal tumors with the same mutation. Now, European and U.S. teams have independently pinpointed epidermal growth factor receptor as the likely missing link in these colorectal cancers, suggesting treatments combining inhibitors of both targets could be more effective than monotherapy.^{1,2} Both groups are independently planning clinical trials with such combinations.

A group led by René Bernards, professor and head of molecular carcinogenesis at **The Netherlands Cancer Institute**, used an RNAi-based genetic screen in human mutant colorectal cancer cells to identify kinases whose knockdown synergized with inhibition of the V600E mutant form of BRAF.

A team led by Jeffrey Engelman used phospho-kinase arrays to identify activation of kinase signaling during BRAF(V600E) inhibition in human colorectal cancer cells. Kinases phosphorylated by the arrays showed which pathways became activated to potentially circumvent BRAF inhibition. Engelman is director of the Center for Thoracic Cancers at the **Massachusetts General Hospital Cancer Center** and assistant professor of medicine at **Harvard Medical School**.

Both teams' experiments pointed to epidermal growth factor receptor (EGFR) as a contributor to BRAF resistance in colorectal cancer and thus as a target to restore the effects of BRAF inhibitors. Previous clinical trials of EGFR inhibitors alone have shown limited benefit in BRAF-mutant colon cancer, which had led researchers to set their sights on targets other than EGFR.³⁻⁵

Bernards' group showed that BRAF-mutant colorectal cancer cell lines did not have a significant response to BRAF or EGFR inhibitors alone. BRAF inhibitor monotherapy also led to higher levels of phosphorylated EGFR than no treatment. Activated EGFR then renewed cell growth through the phosphoinositide 3-kinase (PI3K)-protein kinase B (PKB; PKBA; AKT; AKT1) pathway.

Cells treated with Zelboraf plus an EGFR inhibitor—Erbixub cetuximab, Iressa gefitinib or Tarceva erlotinib—showed greater apoptosis and less viability than cells treated with Zelboraf alone.

In colorectal cancer xenograft mice, a Zelboraf analog plus Erbixub or Tarceva decreased tumor growth compared with any of the molecules as monotherapy, suggesting the combination could help treat BRAF-mutant cancers.

**EDITORIAL****Editor-in-Chief:** Karen Bernstein, Ph.D.**Managing Editor:** Gaspar Taroncher-Oldenburg, Ph.D.**Executive Editor:** Steve Edelson**Senior Editors:** Tracey Baas, Ph.D.; Joanne Kotz, Ph.D.**Writers:** Aaron Bouchie; Chris Cain, Ph.D.; Michael Flanagan; Tim Fulmer, Ph.D.; Michael J. Haas; Stephen Hansen; Kai-Jye Lou; Lauren Martz; Lev Osheroovich, Ph.D.; Steve Usdin**Research Director:** Walter Yang**Research Manager:** Kevin Lehnbeuter**Production Editors:** Brandy Cafarella; Sabina Eberle; Carol Evangelista**Copy Editor:** Nicole DeGennaro**Editorial Assistant:** Mark Zipkin**Design:** Claudia Bentley; Miles DaviesFor inquiries, contact editorial@scibx.com**PUBLISHING****Publisher:** Peter Collins, Ph.D.**Associate Publishers:** Gaspar Taroncher-Oldenburg, Ph.D.; Eric Pierce**Marketing:** Sara Girard; Rosy Rogers**Technology:** Anthony Barrera; Julia Kulikova**Sales:** Ron Rabinowitz; Dean Sanderson; Tim Tulloch**OFFICES****BioCentury Publications, Inc.**San Francisco
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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Data were published in *Nature*. The team included researchers from The Netherlands Cancer Institute, the **University of Torino**, the **Italian Foundation for Cancer Research Institute of Molecular Oncology** and the **Catalan Institute of Oncology**.

Engelman's group showed that Zelboraf plus Iressa led to sustained inhibition of MAPK (ERK), which sits downstream of EGFR, and decreased viability in BRAF-mutant colorectal cancer cell lines. The combination also led

to tumor regression in xenograft mice. In contrast, Zelboraf alone led to incomplete suppression of ERK and renewed cell growth.

The team also studied patient biopsies and found that 60% of BRAF-mutant colorectal cancer samples had high levels of activated EGFR, whereas only 18% of BRAF-mutant melanoma biopsies had high levels of activated EGFR.

Results were published in *Cancer Discovery*. The team included researchers from Massachusetts General Hospital Cancer Center, Harvard Medical School, **Tufts Medical Center, Massachusetts General Hospital and Genentech Inc.**

Zelboraf is marketed to treat metastatic melanoma. The drug is in Phase II testing for thyroid cancer, and a Phase I trial has been completed for colorectal cancers.

Tarceva is marketed by **Astellas Pharma Inc., Chugai Pharmaceutical Co. Ltd.**, and Roche and its Genentech unit to treat non-small cell lung cancer (NSCLC) and pancreatic cancer. Erbitux, from **Eli Lilly and Co., Bristol-Myers Squibb Co.** and **Merck KGaA**, is marked to treat colorectal cancer and head and neck cancer and is under FDA review for NSCLC.

AstraZeneca plc's Iressa is marketed in the EU to treat NSCLC.

Pathway to well

Although both sets of results point to EGFR as a key mediator of resistance to BRAF, each group has its own idea about the mechanism by which EGFR leads to resistance and renewed cell growth (see **Figure 1, "BRAF inhibition leads to EGFR-mediated pathway activation"**).

Bernards' group thinks BRAF inhibition causes increased levels of EGFR phosphorylation. Ultimately, this results in activation of the PI3K-AKT pathway, which renews cell proliferation.

Engelman's team hypothesized that resistance and renewed growth also could be due to EGFR-mediated reactivation of the Ras-Raf-MEK-ERK pathway.

Regardless of the precise mechanism, both teams' findings provide a rationale for the poor clinical response of BRAF(V600E)-mutant

"The findings of the two groups are potentially groundbreaking, demonstrating a novel mechanism of intrinsic resistance that is distinct compared to the findings in melanoma, thereby providing a unique opportunity to combine EGFR- and BRAF-targeted agents in clinical trials for BRAF-mutant colorectal cancer."

—Jeffrey Legos,
GlaxoSmithKline plc

Figure 1. BRAF inhibition leads to EGFR-mediated pathway activation.

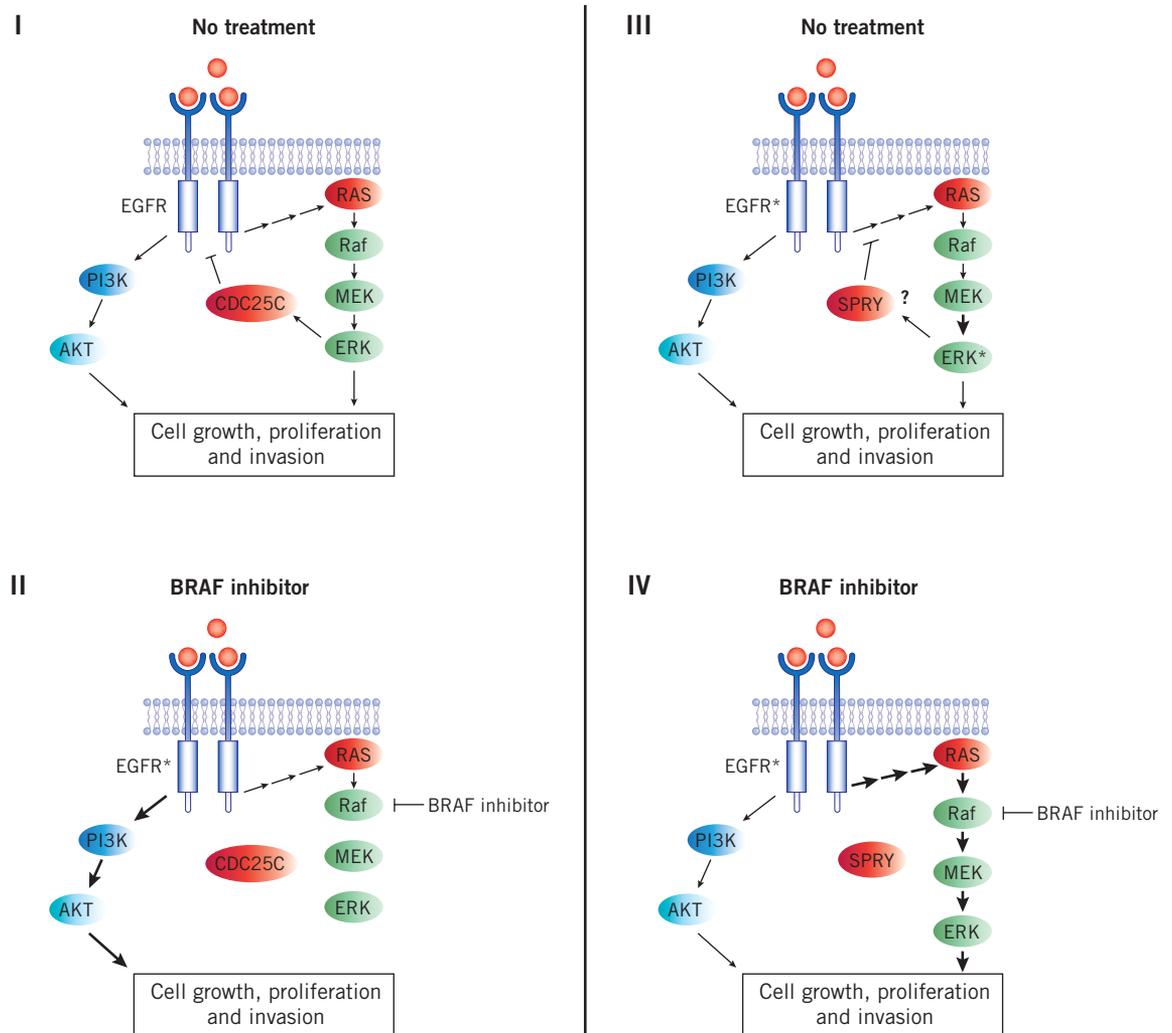
Two groups suggest epidermal growth factor receptor (EGFR)-mediated pathway activation evades BRAF inhibition. Each group has its own idea about the mechanism by which EGFR potentially mediates the pathway activation that leads to renewed cell growth.

Prahallad *et al.* think that in BRAF(V600E)-mutant cancers, normal EGFR signaling results in activation of both the phosphoinositide 3-kinase (PI3K)-protein kinase B (PKB; PKBA; AKT; AKT1) pathway and the Ras-Raf-MEK-MAPK (ERK) pathway. Cell division cycle 25C (CDC25C;

CDC25) binds to and deactivates EGFR (I). The group postulates that BRAF inhibition leads to decreased CDC25C activity and increased EGFR activation (EGFR*). EGFR* then results in greater activation of the PI3K-AKT pathway (bold arrows in II).

Corcoran *et al.* think that in BRAF(V600E)-mutant cancers, EGFR* does not activate Ras, due to an ERK-dependent negative feedback loop—possibly via sprouty (SPRY) proteins (III). The group postulates that BRAF inhibition leads to feedback activation of Ras by EGFR, allowing EGFR to reactivate the Ras-Raf-MEK-ERK pathway (bold arrows in IV).

For either of the proposed EGFR-mediated mechanisms, EGFR inhibitors would potentially block pathway activation and subsequent renewed cell growth.



colorectal cancers to BRAF monotherapy. The data also suggest EGFR levels might be a useful biomarker to decide whether to use monotherapy or combination therapy for any cancer harboring the BRAF(V600E) mutation.

“The preclinical data are very strong, and it is quite feasible to move into clinical trials,” said Engelman. “Because BRAF and EGFR inhibitors are already FDA approved and two separate labs have seen the same effects, I think the translation to clinical trials will be pretty straightforward.”

“The interesting thing is that even though EGFR inhibitors alone have shown no clinical benefit in patients with BRAF(V600E)-mutant colorectal cancer, there are no exclusion criteria for these patients—they are still eligible to receive cetuximab,” noted Bernards.

“The findings of the two groups are potentially groundbreaking, demonstrating a novel mechanism of intrinsic resistance that is distinct compared to the findings in melanoma, thereby providing a unique opportunity to combine EGFR- and BRAF-targeted agents in clinical trials for BRAF-mutant colorectal cancer,” said Jeffrey Legos, GlaxoSmithKline plc’s medicines development leader for their BRAF inhibitor, dabrafenib (GSK2118436).

GSK’s dabrafenib is in Phase I/II clinical trials for solid tumors with BRAF mutations and Phase III testing for BRAF-mutant metastatic melanoma. GSK also has an EGFR1 (HER1; ERBB1) and HER2 (EGFR2; ERBB2; neu) inhibitor, Tykerb lapatinib, that is approved for use in HER2-positive metastatic breast cancer and is also in ongoing Phase III trials in gastric cancer and head and neck cancer.

“And although the groups each have their own interesting point of view on the EGFR-mediated mechanism, their common findings give a possible explanation as to why monotherapy had been a bit dismal for BRAF-mutant colorectal cancer, compared to what we

“As to what this could mean for programs at Genentech, the immediate implications would be the potential opportunity to test EGFR inhibitors in combination with other inhibitors aimed at the Ras-Raf-MEK-ERK pathway.”

**—Jeffrey Settleman,
Genentech Inc.**

would have expected based on melanoma data for this class of drug,” added Legos. “Engelman’s EGFR characterization of patient biopsies gives further credibility, bridging what is occurring in the clinic to what was determined in the laboratory.”

“The work provides a preclinical rationale, in a specific context, for BRAF inhibitors to provide benefit in combination with EGFR inhibitors—which is a combination that would not have been obvious based on prior studies of these pathways,” Jeffrey Settleman told *SciBX*.

Settleman, a coauthor on the *Cancer Discovery* manuscript, is senior director of the discovery oncology program at Genentech. Previously, he was professor of medicine at Massachusetts General Hospital.

“As to what this could mean for programs at Genentech, the immediate implications would be the potential opportunity to test EGFR inhibitors in combination with other inhibitors aimed at the Ras-Raf-MEK-ERK pathway,” Settleman said.

Genentech’s MEHD7945A, a humanized IgG1 mAb targeting EGFR and EGFR3 (HER3; ERBB3), is in Phase I testing for metastatic epithelial tumors.

GDC-0973, a MEK inhibitor from Genentech, Roche and **Exelixis Inc.**, is in Phase I trials to treat melanoma and solid tumors.

“The more far-reaching implications would be the need to more fully understand inhibitor-driven pathway feedback and the redundancy in that feedback,” said Settleman. “When one pathway is blocked, what pathway comes up to compensate for that blockade?

Screening strategies, such as those using siRNA or gain of function, are going to help researchers uncover the interplay between different pathways that lead to resistance and more importantly suggest potential target combinations to circumvent that resistance.”

Both Bernard’s and Engelman’s work is unpatented, and both researchers are in discussions with undisclosed parties to start clinical trials of BRAF and EGFR inhibitors to treat mutant colorectal cancers.

Baas, T. *SciBX* 5(6); doi:10.1038/scibx.2012.140

Published online Feb. 9, 2012

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COMPANIES AND INSTITUTIONS MENTIONED

Astellas Pharma Inc. (Tokyo:4503), Tokyo, Japan
AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Bristol-Myers Squibb Co. (NYSE:BMJ), New York, N.Y.
Catalan Institute of Oncology, L’Hospitalet-Barcelona, Spain
Chugai Pharmaceutical Co. Ltd. (Tokyo:4519), Tokyo, Japan
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Harvard Medical School, Boston, Mass.
Italian Foundation for Cancer Research Institute of Molecular Oncology, Milan, Italy
Massachusetts General Hospital, Boston, Mass.
Massachusetts General Hospital Cancer Center, Boston, Mass.
Merck KGaA (Xetra:MRK), Darmstadt, Germany
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University of Torino, Torino, Italy

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Going viral in Parkinson's disease

By Kai-Iye Lou, Staff Writer

University of Cambridge researchers have fused a virus-derived peptide that crosses the blood brain barrier with a neuroprotective RNA to generate a conjugate that can be delivered noninvasively to the brain in Parkinson's disease models.¹ The group is now elucidating the details of the neuroprotective mechanism and determining the smallest RNA domain needed to elicit the effect.

Multiple groups have previously used viral vectors to deliver therapeutic genes directly into the brain in preclinical models of PD, but John Sinclair, a professor of molecular virology at the University of Cambridge, thinks the safety and efficacy issues make it unlikely that such methods will easily translate into the clinic.

"We did not think intracranial injection with viral vectors that encode the RNA would be a viable option because this type of delivery often leads to a long-term loss of expression and the procedure itself is very invasive," he said.

In 2007, Sinclair's group showed that a 2.7 kb noncoding RNA transcript derived from human cytomegalovirus (CMV) interacts with mitochondrial complex 1 (MC-1) and protects neurons from premature death.²

Deficiencies in MC-1 activity are linked to PD, and Sinclair's group hypothesized that the CMV-derived RNA transcript could help protect against this deficiency.

"If we were going to be serious about eventually using this RNA as a therapeutic, we needed to figure out how to effectively deliver it to a patient, perhaps repeatedly," Sinclair told *SciBX*.

A potential solution came from a **Harvard Medical School** team that published in the same year a method to deliver small interfering RNA cargoes across the blood brain barrier using a modified peptide derived from the rabies virus glycoprotein (RVG).³

Sinclair and his team have now fused the neuroprotective domain of their CMV-derived RNA transcript, called p137 noncoding viral RNA, to the modified RVG peptide.

In adult rats, tail vein injection of the conjugate three days prior to or three days after neurotoxin-induced injury of dopaminergic neurons improved postinjury motor performance compared with injection of the same RVG peptide conjugated to a control RNA transcript. The conjugate also prevented neurotoxin-induced loss of MC-1 activity in the mitochondria of dopaminergic neurons, whereas the control conjugate did not.

Results were published in *The Journal of Experimental Medicine*.

"Mitochondrial stress and free radical-induced toxicity are known to contribute to many neurodegenerative diseases," noted Matthew Wood, professor of neuroscience in the Department of Physiology, Anatomy and Genetics at the **University of Oxford**. "This noncoding viral RNA

could in theory be broadly applicable to neurodegenerative diseases that have similar disease mechanisms."

Sinclair agreed. "We also plan to evaluate our conjugate's ability to protect neurons in preclinical models of Alzheimer's and Huntington's disease," he said.

Wood noted that the Cambridge study is the first example he's seen showing that the RVG peptide could be used to deliver a long strand of viral RNA to the CNS, as opposed to delivering only siRNA, which confirms the value of the peptide for delivering RNA payloads in general.

p137 is about 800 nucleotides long. In contrast, siRNA molecules are usually less than 25 nucleotides long.

Time is of the essence

Sinclair said his group is now trying to determine the minimum domain of the p137 RNA that can still protect neurons from premature death.

The group also is studying the mechanism by which p137 RNA and MC-1 interactions protect the enzyme's activity.

Perhaps most importantly, Sinclair said the team will need to determine how late the conjugate can be delivered during the course of neurodegeneration before its neuroprotective effects are lost.

"What would clearly be a crucial complement to the development of our conjugate in Parkinson's is developing methods to diagnose the disease earlier," he

told *SciBX*. "Because our molecule is protective and prevents further degeneration, earlier diagnosis would help maximize the number of neurons that are still functioning when treatment begins."

Indeed, Wood noted that in PD, the majority of dopaminergic neurons have already degenerated by the time symptoms manifest.

He also said it will be important to determine the conjugate's duration of effect and to explore modifications that increase the stability of the RNA payload.

The University of Cambridge has filed for a patent covering the use of the viral nucleic acids to treat neurodegenerative diseases. The work is available for licensing from **Cambridge Enterprise Ltd.**, the university's technology transfer arm.

Lou, K.-J. *SciBX* 5(6); doi:10.1038/scibx.2012.141

Published online Feb. 9, 2012

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COMPANIES AND INSTITUTIONS MENTIONED

Cambridge Enterprise Ltd., Cambridge, U.K.
Harvard Medical School, Boston, Mass.
University of Cambridge, Cambridge, U.K.
University of Oxford, Oxford, U.K.

HUS and them

By Lauren Martz, Staff Writer

Two groups have identified new strategies to treat or prevent hemolytic uremic syndrome, a potentially lethal complication of *Escherichia coli* infection. A team at the **University of Toronto** has suggested that the marketed CXC chemokine receptor 4 antagonist Mozobil plerixafor could be repurposed for the indication, whereas **Carnegie Mellon University** researchers have found that the natural metal ion manganese could neutralize the toxin that causes the condition.^{1,2}

Hemolytic uremic syndrome (HUS) is caused by the release of Shiga toxins from pathogenic bacteria such as the O157:H7 strain of *E. coli* and triggers symptoms such as anemia, thrombocytopenia and acute renal injury.

There are no available tests to determine whether a patient with gastrointestinal *E. coli* infection will develop HUS, and there are no therapeutics that actually neutralize the toxin. Current treatments are palliative and include dialysis, blood transfusions and corticosteroids. The mortality rate is 5%–7%, and many patients who do recover have long-term renal problems.³

Alexion Pharmaceuticals Inc.'s Soliris eculizumab is approved to treat atypical HUS, a rare genetic form of the disease that is not caused by Shiga toxins. The company is running a Phase II trial of the drug in Shiga toxin–driven HUS and declined to comment.

Now, Philip Marsden and colleagues have used genetic studies to identify the specific genes that are dysregulated by exposure to Shiga toxins. Marsden is professor of medicine and director of nephrology at the University of Toronto and chair in medical research at **St. Michael's Hospital**.

The team's genetic profiling studies showed that the toxin led to greater expression of genes encoding CXC chemokine receptor 4 (CXCR4; NPY3R), CXCR7 and chemokine CXC motif ligand 12 (CXCL12; SDF-1) than expression in untreated controls. These data suggested antagonizing the chemokine pathway could prevent the toxin's effects.

CXCR4 and the chemokine pathway components are known primarily as players in vascular development and maintenance, as well as stem cell homing to the bone marrow.

In mice, Shiga toxin exposure caused weight loss and acute renal failure compared with no exposure. The toxin also altered *Cxcr4*, *Cxcr7* and *Cxcl12* mRNA and protein levels in tissues including the thymus, heart, liver and kidney.

Following exposure to the toxin, 42.3% of mice treated with 10 µg/g of Mozobil daily for 10 days beginning 1 day after toxin exposure survived compared with 23.1% of untreated animals. The drug also improved kidney function compared with control.

Sanofi markets Mozobil to increase mobilization of hematopoietic stem cells to the bloodstream for collection and autologous transplantation in patients with multiple myeloma (MM) or non-Hodgkin's lymphoma (NHL). The pharma declined to comment.

Maria Victoria Ramos, professor of immunology at the **National Academy of Medicine's** Institute of Experimental Medicine, told *SciBX* the data reveal Mozobil has therapeutic and prophylactic potential in HUS.

"One point to be considered about this antagonist is that even though it is injected one day after Shiga toxin treatment, it still reduces renal damage. Considering that there is a period of time between when children are intoxicated with Shiga toxin *E. coli* and when they develop HUS, an effective drug within that window could reduce the risk of disease," she said.

The team also showed that CXCL12 levels were higher in plasma samples from children infected with *E. coli* O157:H7 who progressed to HUS than those in patients whose infections cleared without complication. Levels of the protein were greater in the blood prior to

HUS symptoms, suggesting CXCL12 could be used as a biomarker and that the pathway could be targeted to prevent HUS before the onset of symptoms.

The findings were published in *The Journal of Clinical Investigation*. The paper also included researchers from **The Hospital for Sick Children**, the **University of Iowa Carver College of Medicine**, **Beth Israel Deaconess Medical Center** and the **Washington University in St. Louis School of Medicine**.

Marsden now wants to confirm the findings during an *E. coli* outbreak. He said

that if they could measure CXCL12 levels in real time during an *E. coli* outbreak to confirm the findings, his team would have a strong case for a plerixafor trial in patients with Shiga toxin–producing *E. coli* to see if it prevents or improves HUS cases.

Marsden said the findings are unpatented and unlicensed and that his team is looking for partners.

"Considering that there is a period of time between when children are intoxicated with Shiga toxin *E. coli* and when they develop HUS, an effective drug within that window could reduce the risk of disease."

**—Maria Victoria Ramos,
National Academy of Medicine**

Turning up the metal

Rather than targeting Shiga toxin's mechanism of action like the Toronto team, Carnegie Mellon's Adam Linstedt and Somshuvra Mukhopadhyay have proposed a different approach to prevent HUS: inducing degradation of the toxin by blocking its normal cell-processing pathway.

Linstedt is professor of biological sciences and Mukhopadhyay is a postdoctoral fellow in the Department of Biological Sciences at Carnegie Mellon.

The pair previously showed that manganese, a natural element found in the human body and food sources, causes degradation of proteins involved in the lysosomal pathway of toxins.⁴

Building on that work, Linstedt and Mukhopadhyay monitored the trafficking of Shiga toxin using a fluorescently labeled form of its B subunit. In HeLa cells, the toxin was trafficked from the cell surface to the Golgi apparatus, bypassing the late endosomes and lysosomal degradation pathway. However, when the cells were cultured with manganese, the toxin accumulated in endosome-like structures and was degraded.

In the cells, treatment with manganese protected against a lethal dose of Shiga toxin compared with no treatment. In mice, daily

intraperitoneal administration of manganese beginning 5 days before lethal toxin challenge protected all animals from renal damage and death, whereas all untreated mice died within 96 hours.

Linstedt told *SciBX* that his team does have plans to test whether treatment with manganese beginning once symptoms of HUS have presented could be effective therapeutically, “although our primary interest will be to see if we can effectively treat at the first sign of diarrhea symptoms before the onset of HUS.”

Results were published in *Science*. Linstedt said a patent application has been filed by Carnegie Mellon.

Next steps

Going forward, both strategies could be impeded by a dearth of animal models of *E. coli* infection.

“A major disadvantage for drug developers in the field is that mouse models for *E. coli* infection are not very good to recapitulate diarrheal disease,” said Alfredo Torres, associate professor in the Department of Microbiology and Immunology and the Department of Pathology at **The University of Texas Medical Branch**. “One option could be to see if the results

“A major disadvantage for drug developers in the field is that mouse models for *E. coli* infection are not very good to recapitulate diarrheal disease.”

**—Alfredo Torres,
The University of Texas
Medical Branch**

hold true in humanized mice. These mice could give a better indication of how the treatments will function in humans.”

Torres said another challenge of treating Shiga toxin “is that drug delivery is difficult. It will be ideal to target the toxin within the cell—the challenge is to get a high enough concentration of the drug inside the cells without side effects.”

Linstedt agreed. “High-level exposure of manganese is a known hazard, causing neurological defects especially after prolonged exposure. For treatment, the time of exposure and dose must be minimized,” he said.

Thus, he told *SciBX* the next steps for his team include optimizing the delivery and dosage of manganese in the mouse models.

He suggested that using manganese as part of a combination therapy might allow a lower dose of the ion for therapeutic efficacy. “The main motivation would be to combine manganese with antibiotic therapy so that both the bacteria that spread the toxin and the toxin itself

are neutralized. In theory this might shorten the time period of the treatment so it could also reduce the total exposure of a patient to manganese,” he said.

However, Torres noted that antibiotics are contraindicated for the indication. He said that antibiotic-induced bacterial death induces the release of the toxin and can increase the toxin load in a patient.

Linstedt suggested that developing a therapeutic against manganese’s target, Golgi integral membrane protein 4 (GOLIM4; GPP130), could be an alternative to using manganese. “Whether it would be safer can’t be known until it is tested,” he said. “It would likely be expensive to develop and produce, especially in comparison to manganese.”

For CXCR4 antagonism, Torres suggested the Canadian team should consider developing a more specific inhibitor. “Although they used an approved drug, we need to know what will happen to patients at the required dose when we block this important pathway.” He added, “The inhibitor can always be optimized by drug design to increase binding or translocation to the cells or perhaps to increase potency, resulting in a lower dose that will be required to target the toxin.”

Martz, L. *SciBX* 5(6); doi:10.1038/scibx.2012.142

Published online Feb. 9, 2012

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e-mail: p.marsden@utoronto.ca
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e-mail: linstedt@andrew.cmu.edu
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COMPANIES AND INSTITUTIONS MENTIONED

Alexion Pharmaceuticals Inc. (NASDAQ:ALXN), Cheshire, Conn.
Beth Israel Deaconess Medical Center, Boston, Mass.
Carnegie Mellon University, Pittsburgh, Pa.
National Academy of Medicine, Buenos Aires, Argentina
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
The Hospital for Sick Children, Toronto, Ontario, Canada
St. Michael’s Hospital, Toronto, Ontario, Canada
University of Iowa Carver College of Medicine, Iowa City, Iowa
The University of Texas Medical Branch, Galveston, Texas
University of Toronto, Toronto, Ontario, Canada
Washington University in St. Louis School of Medicine, St. Louis, Mo.

Doubling down on dopamine

By Lev Osherovich, Senior Writer

Dopamine receptor antagonists are widely used to treat schizophrenia but can cause metabolic side effects such as weight gain and type 2 diabetes. Now, a **Scripps Florida** team has pinpointed a receptor complex in the hypothalamus that may play a role in the increase in appetite caused by the drugs.¹ The challenge now is to design next-generation antipsychotics without the appetite-related side effects.

The receptor complex identified consists of two G protein-coupled receptors—the dopamine D2 receptor and the ghrelin receptor (GHSR)—that were not previously suspected to interact with one another. Indeed, the D2 receptor until recently was thought to exist as a simple monomer.

GHSR responds to ghrelin, a hormone that regulates appetite.

“From the early days, it was thought that these receptors functioned as single proteins, and a great deal of pharmacology was based on that view,” said Graeme Milligan, professor of molecular pharmacology at the **University of Glasgow**.

However, recent studies by Milligan and others have suggested the D2 receptor can form heterodimers with a variety of other receptors, including the dopamine D3 receptor.^{2,3}

“The concept of heterodimeric receptors hasn’t received as much attention as it should, perhaps because it’s such a new idea,” said Daniel Müller, associate professor of psychiatry and head of the pharmacogenomics research clinic at the **University of Toronto**. Müller’s team has identified genetic variants in the D2 receptor and other receptors that influence antipsychotic-induced weight gain.⁴

Milligan said much of the data on heterodimer receptors have come from cell culture models rather than animals, and as a result the physiological significance of these complexes has been unclear.

“These dimeric receptors generate distinctive signals, but so far there hasn’t been much evidence of their effect on behavior,” said Milligan. “Companies are being very careful about dipping their toes into this area.”

Now, a team led by Roy Smith, chairman and professor of metabolism and aging at Scripps Florida, has evidence that such dimers do have a therapeutically relevant function. The team used cell culture and mouse studies to show that a D2 receptor–GHSR heterodimer works in the hypothalamus to regulate appetite.

Complex receptor

Smith said his team’s work stems from prior studies of interactions between GHSR and dopamine D1 receptor.

“Several years ago we reported that dopamine receptor D1 and ghrelin receptor are coexpressed” in the same neurons, said Smith. “We showed that those receptors can form heterodimers, but there

was skepticism whether these heterodimers had any physiological function *in vivo*. We really had to demonstrate that dopamine receptor heterodimers are formed in native tissue.”

The D1 receptor is widely expressed in the brain and is not thought to be directly involved in schizophrenia, although it plays a role in other neurological disorders including Parkinson’s disease (PD).⁵

In the new work, Smith’s team tested whether murine Ghsr and D2 receptor could interact in hypothalamic neurons. The team used *in situ* fluorescence methods to show that the two receptors colocalized in hypothalamic neurons in brain slices and physically interacted when transfected together in cell culture.

“D2 receptor has a conflict of interest. If you agonize it, it blocks weight gain, but this could induce psychosis. It may be the heterodimeric D2 receptor–GHSR could be agonized without taking away the effect of antipsychotics.”

—Daniel Müller,
University of Toronto

The team then showed that Ghsr increases the effect of agonizing the D2 receptor. Hypothalamic neurons transfected with both the D2 receptor and Ghsr had a stronger response to a D2 receptor agonist than cells transfected with only the D2 receptor. Pharmacological blockade of Ghsr decreased the effectiveness of D2 receptor agonists compared with no blockade.

The team next tested the physiological significance of the D2 receptor–Ghsr complex. Wild-type animals treated with a D2 receptor agonist had lower food intake than vehicle-treated controls. However, in Ghsr knockouts or wild-type mice treated with a Ghsr antagonist, the D2 receptor agonist did not decrease appetite compared with that seen in wild-type mice treated only with the D2 receptor agonist.

Results were published in *Neuron*.

Compound pharmacology

The findings suggest that if a D2 receptor–GHSR complex exists in humans, it is likely to mediate the effects of dopamine on appetite.

Smith, Milligan and Müller agreed that the next step is to identify compounds that selectively block the D2 receptor–GHSR complex and other D2 receptor-containing heterodimers. But the three researchers had different ideas about what kind of compounds would be most therapeutically useful.

“In the long term, if indeed there are ghrelin receptor heterodimers with D1 receptor and D2 receptor in humans, we could specifically design antagonists to target them,” said Smith. “We are submitting a grant proposal to screen for compounds that selectively antagonize these complexes.”

Smith wants to use such compounds to test whether GHSR–D1 receptor or GHSR–D2 receptor dimers play a role in neurological diseases.

Milligan suspects antagonizing the D2 receptor–GHSR complex is likely to elicit excessive appetite, which would not be desirable for therapeutics. He thinks it would be best to avoid hitting GHSR-containing receptors altogether and instead hit other forms of D2 receptor, such as the D2 receptor–D3 receptor complex that his team identified.

“All conventional antipsychotics are antagonists of D2 receptor, but many antipsychotics are just as effective at the D3 receptor,” said Milligan. “You could overcome some of the side effects of these drugs

by getting a selective compound for the [D3 receptor-containing] heteromeric complex.”

Milligan is collaborating with **Servier** to test the company’s preclinical dopamine receptor antagonists for specific activity against the D2 receptor–D3 receptor complex his team discovered.

Finally, Müller thinks the best bet could be a partial or inverse agonist of D2 receptor–GHSR that would be antagonistic with respect to psychosis but neutral or perhaps agonistic with respect to the receptor complex’s role in suppressing appetite. “D2 receptor has a conflict of interest,” he said. “If you agonize it, it blocks weight gain, but this could induce psychosis. It may be the heterodimeric D2 receptor–GHSR could be agonized without taking away the effect of antipsychotics.”

Another open question is whether the D2 receptor–GHSR complex plays a role in the broader metabolic effects of ghrelin.

Peter DiStefano, CSO of **Elixir Pharmaceuticals Inc.**, noted that the hypothalamus integrates signals from multiple hormonal pathways involved in sensing energy levels and stimulating appetite. Known metabolic players in the hypothalamus include the peptide hormones ghrelin, orexin, leptin and proopiomelanocortin (POMC), but until now dopamine, which is a small molecule, has not been considered a prime target in metabolism.

“I’d like to see a lot more evidence that targeting dopaminergic signaling” can affect body weight, said DiStefano. He suggested a

side-by-side comparison in mice of dopamine receptor agonists and GHSR antagonists on food intake and long-term body weight.

Elixir has GHSR antagonists in preclinical development for obesity and metabolic syndrome and a GHSR agonist that has completed preclinical testing for postoperative ileus and diabetic gastroparesis.

Results of the *Neuron* study are not patented.

Osherovich, L. *SciBX* 5(6); doi:10.1038/scibx.2012.143
Published online Feb. 9, 2012

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COMPANIES AND INSTITUTIONS MENTIONED

Elixir Pharmaceuticals Inc., Cambridge, Mass.
Scripps Florida, Jupiter, Fla.
Servier, Neuilly-sur-Seine, France
University of Glasgow, Glasgow, U.K.
University of Toronto, Toronto, Ontario, Canada

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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				
Cancer	Not applicable	<i>In vitro</i> studies suggest gold-containing indole analogs could help treat cancer. Chemical synthesis and <i>in vitro</i> testing of (phosphine)gold indole analogs identified two lead compounds that induced apoptosis in human breast cancer, colorectal cancer, cervical cancer and leukemia cell lines at low micromolar IC ₅₀ values. In the cervical cancer cell line, each lead compound plus irradiation decreased cell viability compared with irradiation or vehicle alone. Ongoing work includes testing the compounds in animal models of cancer. SciBX 5(6); doi:10.1038/scibx.2012.144 Published online Feb. 9, 2012	Patented by Case Western Reserve University; available for licensing or partnering	Craig, S. <i>et al. J. Med. Chem.</i> ; published online Jan. 30, 2012; doi:10.1021/jm2005942 Contact: Anthony Joseph Berdis, Case Western Reserve University, Cleveland, Ohio e-mail: ajb15@cwru.edu Contact: Thomas Gerald Gray, same affiliation as above e-mail: tgray@case.edu
Cancer	Tryptophan 2,3-dioxygenase (TDO2; TDO)	Mouse studies suggest inhibiting TDO could help treat cancer. In mice, injection of Tdo-expressing mouse tumor cells increased tumor progression compared with injection of non-Tdo-expressing tumor cells. In mice injected with the Tdo-expressing tumor cells, a TDO inhibitor decreased tumor progression compared with no treatment. Next steps include validating the effects of TDO inhibition in additional preclinical models and screening for a stable TDO inhibitor that could be advanced into clinical testing. SciBX 5(6); doi:10.1038/scibx.2012.145 Published online Feb. 9, 2012	Patent applications filed; Ludwig Institute for Cancer Research is in discussions to license findings to a spinoff of the institute	Pilotte, L. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Jan. 30, 2012; doi:10.1073/pnas.1113873109 Contact: Benoît J. Van den Eynde, Université Catholique de Louvain, Brussels, Belgium e-mail: benoit.vandeneynde@bru.licr.org
Chronic myelogenous leukemia (CML)	Janus kinase-2 (JAK-2); signal transducer and activator of transcription 5 (STAT5)	Cell culture and mouse studies suggest JAK-2 inhibitors exert anti-CML effects via off-target activity at STAT5. JAK-2 has been implicated as a target in CML because the kinase activates STAT5, a key driver of leukemia. However, in a mouse model of CML, deletion of <i>Jak-2</i> had no effect on survival compared with normal <i>Jak-2</i> expression. In leukemia cell lines treated with a panel of JAK-2 inhibitors known to decrease leukemia cell growth, deletion of <i>Jak-2</i> did not affect IC ₅₀ values, suggesting the compounds work via off-target effects on STAT5. Next steps include developing therapeutics that directly target STAT5. Jakafi, an oral JAK-1 and JAK-2 inhibitor from Incyte Corp. and Novartis AG, is approved to treat myeloproliferative disorders and is in Phase II trials to treat relapsed and refractory leukemia, including CML. At least seven other companies have compounds that inhibit JAK-2 in Phase II trials or earlier to treat various indications including myeloproliferative disorders. SciBX 5(6); doi:10.1038/scibx.2012.146 Published online Feb. 9, 2012	Unpatented; licensing status not applicable	Hantschel, O. <i>et al. Nat. Chem. Biol.</i> ; published online Jan. 29, 2012; doi:10.1038/nchembio.775 Contact: Giulio Superti-Furga, Austrian Academy of Sciences, Vienna, Austria e-mail: gsuperti-furga@cemmm.oeaw.ac.at Contact: Veronika Sexl, University of Veterinary Medicine Vienna, Vienna, Austria e-mail: veronica.sexl@vetmeduni.ac.at

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Colorectal cancer	BRAF; epidermal growth factor receptor (EGFR)	<p>Two independent studies in mice suggest combining EGFR inhibitors with BRAF inhibitors could help treat BRAF-mutant colorectal cancers that are unresponsive to BRAF inhibitors. In mice with human mutant BRAF colorectal tumors, Zelboraf vemurafenib plus the EGFR inhibitors Tarceva erlotinib or Erbitux cetuximab decreased tumor growth compared with either drug alone. Next steps for both teams could include conducting clinical trials of the combination therapy in BRAF(V600E)-mutant colorectal cancer and identifying other BRAF-mutant cancers.</p> <p>Zelboraf from Daiichi Sankyo Co. Ltd. and partner Roche is approved to treat metastatic melanoma and is in Phase I trials for thyroid cancer and colorectal cancer.</p> <p>Tarceva from Astellas Pharma Inc. and partners Chugai Pharmaceutical Co. Ltd. and Roche is marketed to treat non-small cell lung cancer (NSCLC) and pancreatic cancer.</p> <p>Erbitux from Eli Lilly and Co., Bristol-Myers Squibb Co. and Merck KGaA is marketed to treat colorectal cancer and head and neck cancer, and is under FDA review for NSCLC (<i>see Bringing on the BRAF, page 1</i>).</p> <p>SciBX 5(6); doi:10.1038/scibx.2012.147 Published online Feb. 9, 2012</p>	Findings for both studies unpatented; licensing status not applicable	<p>Prahallad, A. <i>et al. Nature</i>; published online Jan. 26, 2012; doi:10.1038/nature10868 Contact: René Bernards, The Netherlands Cancer Institute, Amsterdam, the Netherlands e-mail: r.bernards@nki.nl</p> <p>Corcoran, R.B. <i>et al. Cancer Discov.</i>; published online Jan. 16, 2012; doi:10.1158/2159-8290.CD-11-0341 Contact: Jeffrey A. Engelman, Massachusetts General Hospital Cancer Center, Boston, Mass. e-mail: jengelman@partners.org</p>
Leukemia	Myeloid-lymphoid or mixed-lineage leukemia (MLL; HRX); multiple endocrine neoplasia I (MEN1; Menin)	<p><i>In vitro</i> studies identified thienopyrimidine-based MEN1 inhibitors that could help treat leukemias. In a panel of human MLL cell lines, inhibitors that blocked the interaction between MEN1 and MLL decreased cell proliferation and expression of target genes and increased apoptosis compared with vehicle. Ongoing studies include developing more potent compounds that will be tested in animal models of MLL.</p> <p>SciBX 5(6); doi:10.1038/scibx.2012.148 Published online Feb. 9, 2012</p>	Patent application filed; available for licensing	<p>Grembecka, J. <i>et al. Nat. Chem. Biol.</i>; published online Jan. 29, 2012; doi:10.1038/nchembio.773 Contact: Tomasz Cierpicki, University of Michigan, Ann Arbor, Mich. e-mail: tomaszc@umich.edu Contact: Jolanta Grembecka, same affiliation as above e-mail: jolantag@umich.edu</p>
Leukemia	WEE1 tyrosine kinase (WEE1)	<p>Patient sample and cell culture studies suggest combining WEE1 inhibitors with cytarabine could help treat leukemia. In leukemia cell lines, cytarabine plus the WEE1 inhibitor MK-1775 increased apoptosis compared with either compound alone. Next steps include running a clinical trial of MK-1775 plus cytarabine in leukemia patients. Merck & Co. Inc.'s MK-1775 is in Phase II testing to treat solid tumors.</p> <p>Cytarabine is a generic chemotherapeutic used to treat hematological cancers.</p> <p>SciBX 5(6); doi:10.1038/scibx.2012.149 Published online Feb. 9, 2012</p>	Unpatented; licensing status not applicable	<p>Tibes, R. <i>et al. Blood</i>; published online Jan. 20, 2012; doi:10.1182/blood-2011-07-367557 Contact: Raoul Tibes, Mayo Clinic, Scottsdale, Ariz. e-mail: tibes.raoul@mayo.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Lung cancer	Anaplastic lymphoma kinase (ALK); epidermal growth factor receptor (EGFR); stem cell factor receptor tyrosine kinase (c-Kit; KIT; CD117)	<p>Patient and cell culture studies suggest targeting EGFR and KIT signaling pathways could help treat lung cancers resistant to ALK inhibitors. In samples from 18 patients resistant to Xalkori crizotinib, 17 showed EGFR activation and 2 samples showed KIT amplification. In Xalkori-resistant human lung cancer cell lines, Xalkori plus an EGFR or KIT inhibitor decreased proliferation compared with Xalkori alone. Next steps include identifying additional mechanisms of Xalkori resistance and determining how they influence resistance to next-generation ALK inhibitors.</p> <p>Pfizer Inc. markets Xalkori, a dual c-Met receptor tyrosine kinase and ALK inhibitor, to treat non-small cell lung cancer (NSCLC).</p> <p>At least four other companies have ALK inhibitors in Phase I/II testing or earlier to treat cancer.</p> <p>SciBX 5(6); doi:10.1038/scibx.2012.150 Published online Feb. 9, 2012</p>	Work unpatented; licensing status not applicable	<p>Katayama, R. <i>et al. Sci. Transl. Med.</i>; published online Jan. 25, 2012; doi:10.1126/scitranslmed.3003316</p> <p>Contact: Jeffrey A. Engelman, Massachusetts General Hospital Cancer Center, Boston, Mass. e-mail: jengelman@partners.org</p>
Non-small cell lung cancer (NSCLC)	Guanine nucleotide binding protein β -polypeptide 2-like 1 (GNB2L1; RACK1)	<p>Patient sample and mouse studies suggest inhibiting RACK1 could help treat NSCLC. RACK1 levels were higher in 48 of 63 samples from patients with NSCLC than in samples from matched normal tissue. In a mouse xenograft model of NSCLC, small interfering RNA against RACK1 lowered tumor growth and metastasis compared with control siRNA. Next steps could include screening for small molecule RACK1 inhibitors.</p> <p>SciBX 5(6); doi:10.1038/scibx.2012.151 Published online Feb. 9, 2012</p>	Patent and licensing status unavailable	<p>Shi, S. <i>et al. J. Biol. Chem.</i>; published online Jan. 19, 2012; doi:10.1074/jbc.M111.315416</p> <p>Contact: Dong Xie, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Graduate University of the Chinese Academy of Sciences, Shanghai, China e-mail: dxie@sibs.ac.cn</p>
Prostate cancer	Aldo-keto reductase family 1 member C3 (AKR1C3)	<p><i>In vitro</i> studies identified specific AKR1C3 inhibitors that could help treat castration-resistant prostate cancer. AKR1C3 is upregulated in castration-resistant prostate cancer. In <i>in vitro</i> assays, flufenamic acid-based compounds selectively inhibited AKR1C3 with nanomolar potencies. In a prostate cancer cell line overexpressing AKR1C3, the lead inhibitor decreased testosterone formation compared with no treatment. Next steps include testing the inhibitors in xenograft models of castration-resistant prostate cancer.</p> <p>SciBX 5(6); doi:10.1038/scibx.2012.152 Published online Feb. 9, 2012</p>	Patent applications filed; disclosed and other undisclosed compounds available for licensing	<p>Adeniji, A.O. <i>et al. J. Med. Chem.</i>; published online Jan. 20, 2012; doi:10.1021/jm201547v</p> <p>Contact: Trevor M. Penning, University of Pennsylvania, Philadelphia, Pa. e-mail: penning@upenn.edu</p> <p>Contact: Jeffrey D. Winkler, same affiliation as above e-mail: winkler@sas.upenn.edu</p>
Prostate cancer	O-Linked N-acetylglucosamine transferase (OGT)	<p>Mouse studies suggest inhibiting OGT could help treat prostate cancer. In a mouse xenograft model of prostate cancer, small hairpin RNA against OGT decreased bone metastasis compared with control shRNA. Next steps include identifying OGT inhibitors.</p> <p>SciBX 5(6); doi:10.1038/scibx.2012.153 Published online Feb. 9, 2012</p>	Patented; available for licensing and partnering	<p>Lynch, T.P. <i>et al. J. Biol. Chem.</i>; published online Jan. 24, 2012; doi:10.1074/jbc.M111.302547</p> <p>Contact: Mauricio J. Reginato, Drexel University College of Medicine, Philadelphia, Pa. e-mail: mauricio.reginato@drexelmed.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cardiovascular disease				
Ischemia/reperfusion injury	Eukaryotic translation initiation factor 2 α kinase 4 (EIF2AK4; GCN2)	Studies in mice suggest activation of the amino acid starvation response could protect against surgical ischemia/reperfusion injury. In mouse models of hepatic and renal ischemia, a tryptophan-free diet or a complete diet plus the amino acid starvation response activator halofuginone led to less organ injury than a complete diet alone. Next steps could include determining if the amino acid starvation response also protects aged or obese mice from hepatic or renal ischemia.	Patent and licensing status unavailable	Peng, W. <i>et al. Sci. Transl. Med.</i> ; published online Jan. 25, 2012; doi:10.1126/scitranslmed.3002629 Contact: James R. Mitchell, Harvard School of Public Health, Boston, Mass. e-mail: jmitchel@hsph.harvard.edu
Endocrine/metabolic disease				
Dyslipidemia	Diacylglycerol O-acyltransferase-1 (DGAT1)	Rodent and cell culture studies suggest the DGAT1 inhibitor ABT-046 could help treat dyslipidemia. In a human cell line, ABT-046 inhibited triglyceride formation with an IC ₅₀ of 78 nM. In mice receiving a lipid challenge, oral ABT-046 lowered serum triglycerides compared with vehicle ($p < 0.05$). Next steps could include testing ABT-046 in animal models of diabetes. ABT-046 is in preclinical development at Abbott Laboratories for undisclosed indications. Novartis AG's DGAT1 inhibitor, LCQ908, is in Phase II testing in diabetes.	Patent application filed; available for licensing	Souers, A.J. <i>J. Med. Chem.</i> ; published online Jan. 20, 2012; doi:10.1021/jm201524g Contact: Andrew James Souers, Abbott Laboratories, Abbott Park, Ill. e-mail: andrew.souers@abbott.com
Infectious disease				
HCV	Membrane-bound transcription factor peptidase site-1 (MBTPS1; SKI-1)	Cell culture studies suggest inhibiting SKI-1 could help treat HCV infection. In cultured hepatocytes, a small molecule MBTPS1 inhibitor decreased HCV infection and viral titers compared with vehicle control. The inhibitor did not lower HCV RNA levels, suggesting the compound is acting at a late assembly stage of the viral life cycle. Next steps include developing more potent active site-directed SKI-1 inhibitors with better pharmacokinetic properties.	Patent and licensing status undisclosed	Olmstead, A.D. <i>et al. PLoS Pathog.</i> ; published online Jan. 5, 2012; doi:10.1371/journal.ppat.1002468 Contact: François Jean, The University of British Columbia, Vancouver, British Columbia, Canada e-mail: fjean@mail.ubc.ca
Musculoskeletal disease				
Osteoporosis	<i>Pleckstrin homology domain containing family O member 1</i> (PLEKHO1; CKIP-1)	Rat studies suggest bone formation-targeting liposomes loaded with <i>PLEKHO1</i> small interfering RNA could help treat osteoporosis. In normal rats, the peptide (Asp-Ser-Ser) ₆ homed to bone formation surfaces but not to bone resorption surfaces. In rat models of osteoporosis, liposomes linked to the peptide and loaded with siRNA against <i>Plekho1</i> increased bone mineral density compared with <i>Plekho1</i> siRNA-loaded liposomes lacking the peptide or nonliposomal <i>Plekho1</i> siRNA. Future studies could include testing the targeted <i>Plekho1</i> siRNA-loaded liposomes in animal models of periodontitis and other bone loss indications. <i>Plekho1</i> encodes a bone formation-inhibiting protein.	Patent and licensing status unavailable	Zhang, G. <i>et al. Nat. Med.</i> ; published online Jan. 29, 2012; doi:10.1038/nm.2617 Contact: Ling Qin, The Chinese University of Hong Kong, Hong Kong, China e-mail: lingqin@cuhk.edu.hk Contact: Ge Zhang, same affiliation as above e-mail: zhangge@ort.cuhk.edu.hk Contact: Lingqiang Zhang, Beijing Institute of Radiation Medicine, Beijing, China e-mail: zhanglq@nic.bmi.ac.cn

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Neurology				
Alzheimer's disease (AD)	N-Acetyltransferase 8 (NAT8); NAT8B	Human brain tissue and cell culture studies suggest NAT8 and NAT8B could be antagonized to treat AD. In brain tissue from AD patients, compared with healthy control tissue, NAT8 and NAT8B were overexpressed. In a cell culture model of AD, a small molecule inhibitor of NAT8 and NAT8B lowered levels of β -site APP-cleaving enzyme 1 (BACE1), an enzyme known to promote AD pathology, compared with vehicle. Next steps include optimizing NAT8 and NAT8B inhibitors for use in the CNS. SciBX 5(6); doi:10.1038/scibx.2012.158 Published online Feb. 9, 2012	Patent pending; available for licensing	Ding, Y. <i>et al. J. Biol. Chem.</i> ; published online Jan. 20, 2012; doi:10.1074/jbc.M111.310136 Contact: Luigi Puglielli, University of Wisconsin–Madison, Madison, Wis. e-mail: lp1@medicine.wisc.edu
Neurology	Solute carrier family 12 potassium- chloride transporter member 2 (SLC12A2, NKCC1); p75 neurotrophin receptor (p75 NTR)	Studies in mice suggest that bumetanide could help treat nerve injury. In wild-type mice, motor neuron trauma resulted in upregulation of p75 Ntr and cell death, which was blocked with intraperitoneal injection of the SLC12A2 inhibitor bumetanide. Next steps could include testing bumetanide in other models of nerve injury. Only for Children Pharmaceuticals S.A.S. has bumetanide in Phase II testing to treat seizures. SciBX 5(6); doi:10.1038/scibx.2012.159 Published online Feb. 9, 2012	Patent and licensing status unavailable	Shulga, A. <i>et al. J. Neurosci.</i> ; published online Feb. 1, 2012; doi:10.1523/JNEUROSCI.3282-11.2012 Contact: Claudio Rivera, University of Helsinki, Helsinki, Finland e-mail: claudio.rivera@helsinki.fi
Parkinson's disease (PD)	Regulator of G-protein signaling 4 (RGS4)	Mouse studies suggest inhibiting RGS4 could help treat PD. In a mouse model of PD, <i>Rgs4</i> knockout led to less PD deficits compared with normal expression of the gene. Next steps include identifying RGS4 inhibitors and testing the candidates in animal models. SciBX 5(6); doi:10.1038/scibx.2012.160 Published online Feb. 9, 2012	Findings unpatented; licensing status not applicable	Lerner, T.N. & Kreitzer, A.C. <i>Neuron</i> ; published online Jan. 26, 2012; doi:10.1016/j.neuron.2011.11.015 Contact: Anatol C. Kreitzer, University of California, San Francisco, Calif. e-mail: akreitzer@gladstone.ucsf.edu
Rett syndrome	Neurotrophic tyrosine kinase receptor 2 (NTRK2; TrkB)	Mouse studies suggest TrkB agonists could help improve breathing dysfunction in patients with Rett syndrome. Rett syndrome patients and mouse models of Rett syndrome have lower levels of brain-derived neurotrophic factor (BDNF), which increase respiratory frequency. In a mouse model of Rett syndrome, a small molecule agonist of the Bdnf receptor TrkB restored levels of Bdnf and activated TrkB in the brain, and lowered breathing frequency compared with vehicle control. Next steps could include developing a TrkB agonist. SciBX 5(6); doi:10.1038/scibx.2012.161 Published online Feb. 9, 2012	Patent and licensing status unavailable	Schmid, D.A. <i>et al. J. Neurosci.</i> ; published online Feb. 1, 2012; doi:10.1523/JNEUROSCI.0865-11.2012 Contact: David M. Katz, Case Western Reserve University School of Medicine, Cleveland, Ohio e-mail: david.katz@case.edu

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Schizophrenia	Dopamine D2 receptor; ghrelin receptor (GHSR)	<p>Studies in cell culture and in mice suggest a heterodimeric complex of the dopamine D2 receptor and GHSR may be involved in hyperphagia caused by antipsychotic therapy. In cultured primary hypothalamic neurons, the D2 receptor and GHSR were physically associated, and treatment with antagonists of either receptor blocked dopamine receptor-induced neuronal activation compared with vehicle treatment. Ghsr knockout mice receiving a D2 receptor agonist had less food intake than wild-type controls. Next steps include identifying selective modulators of the D2 receptor-GHSR complex and other dopamine receptor complexes and testing their effects in mouse models of schizophrenia.</p> <p>At least 12 companies have D2 receptor antagonists in development and on the market for schizophrenia and other neurological indications (<i>see Doubling down on dopamine, page 8</i>).</p> <p>SciBX 5(6); doi:10.1038/scibx.2012.162 Published online Feb. 9, 2012</p>	Unpatented; licensing status not applicable	<p>Kern, A. <i>et al. Neuron</i>; published online Jan. 26, 2012; doi:10.1016/j.neuron.2011.10.038</p> <p>Contact: Roy G. Smith, Scripps Florida, Jupiter, Fla. e-mail: rgsmith@scripps.edu</p>
Ophthalmic disease				
Retinitis	<i>Retinitis pigmentosa GTPase regulator (RPGR)</i>	<p>Canine studies suggest gene therapy could be used to correct defects in <i>RPGR</i> that lead to retinitis pigmentosa, a hereditary form of vision loss. In a canine model of retinitis pigmentosa, an adeno-associated virus (AAV) vector with a functional copy of human <i>RPGR</i> decreased photoreceptor degeneration and restored retinal function compared with no treatment. Next steps include optimizing gene expression of the construct in preparation for clinical testing.</p> <p>Applied Genetic Technologies Corp. has patents on the AAV vector used in the study and has a related construct in Phase I/II testing for Leber's congenital amaurosis, another hereditary retinal indication.</p> <p>SciBX 5(6); doi:10.1038/scibx.2012.163 Published online Feb. 9, 2012</p>	Patent pending; available for licensing	<p>Beltran, W.A. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Jan. 23, 2012; doi:10.1073/pnas.1118847109</p> <p>Contact: Gustavo D. Aguirre, University of Pennsylvania, Philadelphia, Pa. e-mail: gda@vet.upenn.edu</p> <p>Contact: William A. Beltran, same affiliation as above e-mail: wbeltran@vet.upenn.edu</p>
Pulmonary disease				
Cystic fibrosis (CF)	Cystic fibrosis transmembrane conductance regulator (CFTR)	<p><i>In vitro</i> studies suggest stabilizing two distinct steps of mutant $\Delta F508$ CFTR protein folding could help treat CF. <i>In vitro</i> studies showed the $\Delta F508$ mutation in CFTR disrupts two protein-folding steps and leads to CFTR degradation. Computational and thermodynamic analyses of $\Delta F508$ CFTR identified two sets of suppressor mutations that synergized to correct CFTR folding and restore protein function to wild-type levels. Next steps include screening for compounds that affect each CFTR folding step and identifying which folding step is affected by existing CFTR-targeted compounds.</p> <p>Vertex Pharmaceuticals Inc.'s VX-809, a CFTR corrector, is in Phase II trials to treat $\Delta F508$ CF. Vertex's VX-661, also a CFTR corrector, is in preclinical development to treat $\Delta F508$ CF.</p> <p>SciBX 5(6); doi:10.1038/scibx.2012.164 Published online Feb. 9, 2012</p>	Findings for both studies unpatented; licensing status not applicable	<p>Rabeh, W.M. <i>et al. Cell</i>; published online Jan. 20, 2012; doi:10.1016/j.cell.2011.11.024</p> <p>Contact: Gergely L. Lukacs, McGill University, Montreal, Quebec, Canada e-mail: gergely.lukacs@mcgill.ca</p> <p>Mendoza, J.L. <i>et al. Cell</i>; published online Jan. 20, 2012; doi:10.1016/j.cell.2011.11.023</p> <p>Contact: Philip J. Thomas, The University of Texas Southwestern Medical Center, Dallas, Texas e-mail: philip.thomas@utsouthwestern.edu</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
Assays & screens			
<i>In vitro</i> transmigration assay to model the blood brain barrier (BBB)	An <i>in vitro</i> transmigration assay could be a useful BBB model to help identify new targets and compounds to treat CNS disorders like multiple sclerosis (MS). The assay measures the transmigration of peripheral blood mononuclear cells, while the cells are under shear forces to mimic blood flow, across a layer of human brain microvascular endothelial cells cultured on a filter. In the model, an anti-CXC chemokine receptor 4 (CXCR4; NPY3R) antibody blocked chemokine CXC motif ligand 12 (CXCL12; SDF-1)-induced cell transmigration compared with an IgG isotype control. Next steps could include looking for chemokines and chemokine receptors involved in mediating immune cell invasion of the CNS in MS.	Unpatented; licensing status not applicable	Man, S. <i>et al. Sci. Transl. Med.</i> ; published online Feb. 1, 2012; doi:10.1126/scitranslmed.3003197 Contact: Richard M. Ransohoff, Cleveland Clinic, Cleveland, Ohio e-mail: ransohr@ccf.org
Disease models			
Mouse model of intrahepatic cholangiocarcinoma	A mouse model of cholangiocarcinoma could be used to screen for treatments of the disease. In mice, a conditionally activated mutant <i>K-Ras</i> allele plus a conditional <i>p53</i> knockout allele led to liver tumors that recapitulated human intrahepatic cholangiocarcinoma. In cell lines derived from those mouse tumors, chloroquine-mediated inhibition of autophagy lowered proliferation compared with no treatment. Next steps include characterizing the early stages of cholangiocarcinoma and incorporating additional commonly mutated genes into the model.	Unpatented; licensing status undisclosed	O'Dell, M.R. <i>et al. Cancer Res.</i> ; published online Jan. 20, 2012; doi:10.1158/0008-5472.CAN-11-3596 Contact: Aram F. Hezel, University of Rochester Medical Center, Rochester, N.Y. e-mail: aram_hezel@urmc.rochester.edu

CORRIGENDA AND ERRATA

Erratum: The Distillery: cancer: retinoblastoma

SciBX 5(5); doi:10.1038/scibx.2012.122
Published online Feb. 2, 2012

A Therapeutics item on cancer, highlighting an article by Zhang *et al.*, misstated the name of the compound from Rigel Pharmaceuticals and AstraZeneca. The compound name is fostamatinib (R788).

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