

THIS WEEK**ANALYSIS****COVER STORY****1 Eyeing the inflammasome**

Two academic teams have independently shown that targeting the inflammasome reduces retinal damage in mouse models of age-related macular degeneration. One study favors inhibiting inflammasome activation to treat dry AMD, and those findings are licensed to iVeena Pharmaceuticals. The other approach suggests boosting the inflammasome to treat wet AMD.

TARGETS & MECHANISMS**5 Paradoxical P2X7**

Italian researchers have shown that inhibiting the P2X7 receptor can treat cancer. The findings could open up a new disease area for companies developing P2X7 antagonists to treat pain, multiple sclerosis, inflammatory bowel disease and rheumatoid arthritis.

7 K-Ras in cancer metabolism

Researchers from the Dana-Farber Cancer Institute have identified a glucose metabolism pathway that is activated by the *K-Ras* oncogene in pancreatic cancer. Based on the findings, it may be possible to target proteins in the pathway to block proliferation of K-Ras-driven cancers.

TOOLS**9 PROTAC the protein**

GSK and Yale researchers have announced a collaboration to develop a platform that selectively tags disease-associated proteins with an E3 ubiquitin ligase ligand, thus targeting them to a cell's protein degradation machinery. GSK hopes the platform could be a cornerstone for a new Discovery Performance Unit at the pharma, and the partners are aiming to have proof-of-principle results in cell culture by year end.

THE DISTILLERY**11 This week in therapeutics**

Preventing inflammation-induced heart failure by blocking TLR9; increasing antibiotic efficacy by inhibiting DNA repair; treating pain with cyclic peptide-based B1R antagonists; and more...

16 This week in techniques

Using 2'-F antisense oligonucleotides to modulate splicing; and more...

INDEXES**17 Company and institution index****17 Target and compound index****Eyeing the inflammasome**

By *Tim Fulmer, Senior Writer*

Two academic teams have independently shown that targeting the inflammasome reduces retinal damage in mouse models of age-related macular degeneration.^{1,2} One study favors inhibiting inflammasome activation to treat dry AMD, whereas the other suggests boosting it to treat wet AMD. The approach for treating dry AMD has been licensed to **iVeena Pharmaceuticals Inc.**, and the academics focused on wet AMD are developing their own gene therapy.

Inflammasomes are a family of cytosolic protein complexes that consist of three subunits: one of several nod-like receptor proteins (NLRs), the PYD and CARD domain containing (PYCARD; ASC) protein, and caspase-1 (CASP1). In immune cells, activation of the inflammasome by pathogens triggers release of the proinflammatory cytokines IL-1 β and IL-18, which then recruit effector cells to the site of tissue injury or infection as part of the innate immune response.

The inflammasome and its downstream effector cytokines have been implicated in inflammation, cancer and metabolic disorders.^{3,4} However, until now there had been no evidence that the complex plays a role in AMD and other eye diseases.

The new papers suggest that the NLR family pyrin domain containing 3 (NLRP3; NALP3) inflammasome, which consists of NLRP3, ASC and CASP1, mediates the retinal toxicity of two molecules linked to AMD progression: drusen proteins and *Alu* RNA (see Figure 1, "The inflammasome in age-related macular degeneration"). Moreover, both showed that targeting downstream effectors of the inflammasome reduces retinal damage in mouse AMD models.

To serve and protect

Drusen are deposits of extracellular proteins that form under the retina and are associated with risk for developing AMD. Despite the correlation between AMD and large drusen deposits in the macula, the underlying mechanism of drusen toxicity has remained unclear.

A team of Irish researchers hypothesized that drusen might cause toxicity through activation of the NLRP3 inflammasome because molecular aggregates, crystals and deposits are known to activate the inflammasome in various other diseases. These include cholesterol crystals in atherosclerosis,⁵ islet amyloid peptide aggregates in type 2 diabetes⁶ and uric acid crystals in gout.⁷

Moreover, excess drusen is known to trigger cellular necrosis, a process that also activates the NLRP3 inflammasome.⁸

To test whether drusen activate inflammasome signaling in the eye,



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 Osheroich, Ph.D.; Steve Usdin**Research Director:** Walter Yang**Research Manager:** Kevin Lehnbeuter**Production Editors:** Brandy Cafarella; 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.

Copyright © 2012 Nature Publishing Group ALL RIGHTS RESERVED.

No part of the *SciBX* publication or website may be copied, reproduced, retransmitted, disseminated, sold, distributed, published, broadcast, circulated, commercially exploited or used to create derivative works without the written consent of the Publishers. Information provided by the *SciBX* publication and website is gathered from sources that the Publishers believe are reliable; however, the Publishers do not guarantee the accuracy, completeness, or timeliness of the information, nor do the Publishers make any warranties of any kind regarding the information. The contents of the *SciBX* publication and website are not intended as investment, business, tax or legal advice, and the Publishers are not responsible for any investment, business, tax or legal opinions cited therein.

the team first isolated drusen from donor eyes of deceased patients with AMD and added them to cultured human peripheral blood mononuclear cells (PBMCs), which are immune cells that enter the retina during AMD and express the NLRP3 inflammasome.

In those PBMCs, even low concentrations of drusen triggered a significant increase in production of the two main inflammasome effector cytokines—IL-1 β and IL-18—compared with no drusen ($p<0.0001$). In cultured murine PBMCs, compared with wild-type PBMCs, Nlrp3 knockout led to decreased IL-1 β production following addition of drusen.

Thus, drusen-induced upregulation of IL-1 β and IL-18 in monocytes required activation of the NLRP3 inflammasome.

Next, the researchers looked at the role of inflammasome activation in the development and progression of AMD.

In a mouse model of wet AMD, knockout of *Nlrp3* or *Il-18* led to significantly greater choroidal neovascularization (CNV) volume than that in wild-type controls ($p<0.05$), suggesting that NLRP3 inflammasome activation and IL-18 expression both protected against CNV. Intravitreal injection of an anti-IL-18 antibody also significantly worsened CNV in the mice compared with no treatment ($p=0.0368$).

The researchers hypothesized that IL-18 might protect against wet AMD by downregulating VEGF, which drives excessive blood vessel production in the retina. Indeed, in a murine brain endothelial cell line, recombinant IL-18 significantly lowered Vegf secretion compared with no treatment ($p<0.05$).

Taken together, the findings suggest inflammasome activation in immune cells plays a protective role in wet AMD, whereby drusen trigger the NLRP3 inflammasome to upregulate expression of IL-18. The cytokine, in turn, reduces levels of proangiogenic VEGF.

The authors wrote that “a balance may exist whereby a certain focal amount of drusen is tolerated because of its ability to induce IL-18, which in turn may act as an anti-angiogenic effector.” They concluded that their findings suggest “strategies aimed at producing or delivering IL-18 to the eye may be beneficial in preventing the progression of CNV in the context of wet AMD.” However, “once a critical level of drusen accumulates, its protective role is negated” and disease sets in, they wrote.

Progression to wet AMD occurs in about 10% of cases of the dry form, co-lead author Sarah Doyle told *SciBX*.

Results were published in *Nature Medicine*.

The study was led by Matthew Campbell, a researcher in the Smurfit Institute of Genetics at **Trinity College Dublin**, Doyle, a researcher in the **Trinity Biomedical Sciences Institute**, and Peter Humphries, professor of medical molecular genetics at the Smurfit Institute at Trinity College. Joe Hollyfield, professor of ophthalmology at the **Cleveland Clinic Lerner College of Medicine of Case Western Reserve University**, isolated the drusen used in the experiments.

The dry path

In the second paper, a group headed by Jayakrishna Ambati, professor of physiology and vice chair of ophthalmology and visual sciences at the

“Strategies aimed at producing or delivering IL-18 to the eye may be beneficial in preventing the progression of CNV in the context of wet AMD.”

—Doyle et al.,
Trinity College Dublin

Figure 1. The inflammasome in age-related macular degeneration. Two groups have independently found that the inflammasome and IL-18 play a key role in the development and progression of age-related macular degeneration (AMD).

The inflammasome is a cytosolic, multiprotein complex that triggers the innate immune response to microbial toxins as well as to endogenous proteins, lipids and oligonucleotides.

In a paper published in *Nature Medicine*, Doyle *et al.* showed that protein aggregates in the macula known as drusen activated the inflammasome in monocytes [a(1) and b(1)] to trigger the local production of proinflammatory cytokine IL-18 [c(1)]. The result was downregulation of proangiogenic VEGF and reduced eye damage [d(1) and e(1)] in a mouse model of wet AMD.

In a paper published in *Cell*, Tarallo *et al.* showed that *Alu* RNA in the macula plus reactive oxygen species (ROS) activated the inflammasome in retinal pigment epithelial (RPE) cells [a(2) and b(2)] to trigger production of IL-18 [c(2)]. The result was activation of myeloid differentiation primary response gene 88 (MYD88), increased RPE degeneration and eye damage [d(2) and e(2)] in a mouse model of geographic atrophy, an advanced form of dry AMD.

Doyle *et al.* are now developing adeno-associate virus (AAV) vector-mediated delivery of pro-IL-18 gene therapy to enhance IL-18 activity in the eye and treat wet AMD, whereas Tarallo *et al.* are developing MYD88 inhibitors to block IL-18 activity and treat dry AMD.

University of Kentucky, found that inflammasome activation worsened retinal damage in geographic atrophy, an advanced form of dry AMD.

The Kentucky researchers followed up on their own 2011 *Nature* paper that showed that deficiency in the microRNA-processing enzyme dicer 1 ribonuclease type III (DICER1) led to accumulation of *Alu* RNA molecules in the retinal pigment epithelium (RPE). The result was geographic atrophy and retinal degeneration.⁹

Based on those findings, Ambati and colleagues had originally planned to reduce levels of toxic *Alu* RNA in the retina using one of two approaches. “We reasoned we could either use *Alu* antisense therapy to directly lower *Alu* RNA levels or use *DICER1* gene therapy to boost *DICER1* levels, which would increase processing of *Alu* RNA and lower *Alu* RNA levels,” said corresponding author Ambati.

“However, we eventually abandoned both approaches. It proved too difficult to titrate the antisense and gene therapies into the back of the eye without causing dysregulation of RNA processing or unacceptable levels of inflammation,” Ambati told *SciBX*.

Thus, Ambati and colleagues looked downstream of *Alu* RNA. The challenge was determining which protein *Alu* RNA initially bound to trigger the toxicity cascade.

The team used a series of knockout mice and cell lines to successively eliminate potential RNA-binding targets such as toll-like receptors (TLRs) and RNA-sensing proteins. For each knockout line, the group

compared levels of *Alu* RNA-mediated RPE degeneration with those in wild-type controls, eliminating from further consideration any protein whose knockout did not result in less degeneration than the wild-type comparator.

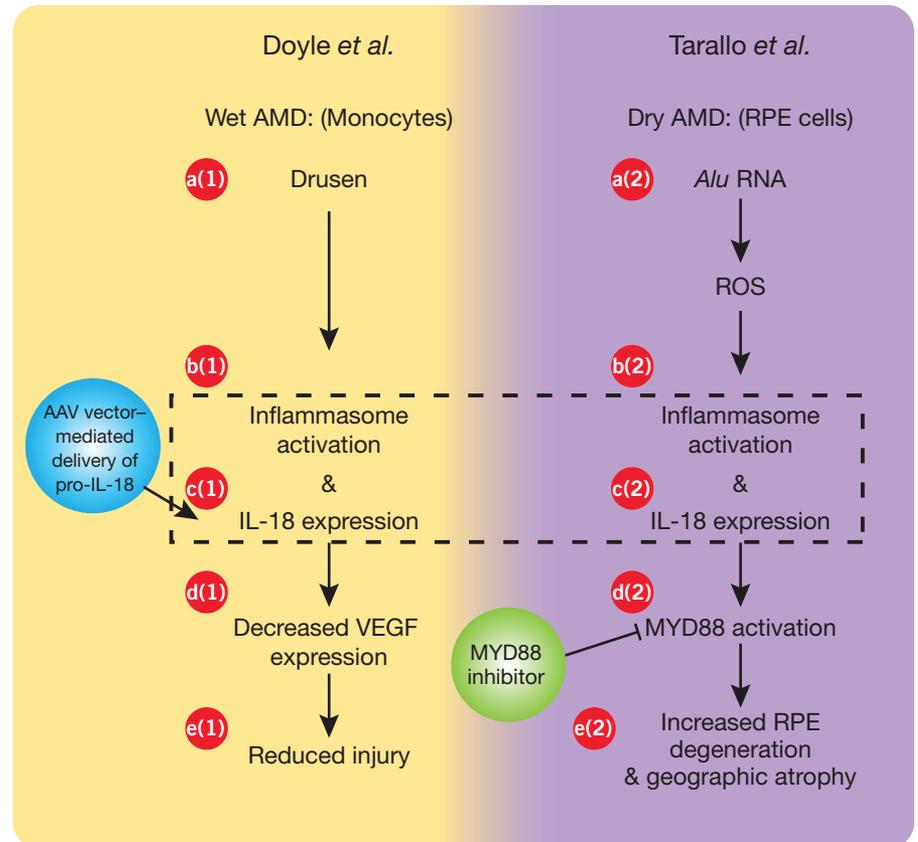
By process of elimination, the researchers arrived at the NLRP3 inflammasome and its downstream effector myeloid differentiation primary response gene 88 (MYD88).

Myd88 knockout mice showed no *Alu* RNA-mediated RPE degeneration compared with wild-type controls, nor did mice with knockout of *Nlrp3* or *Asc*. In wild-type mice, intravitreal delivery of a MYD88 peptide inhibitor prevented RPE degeneration.

Finally, to determine whether the toxicity mechanism had any relevance to patients with AMD, the researchers looked at whether human eyes with *Alu* RNA-associated geographic atrophy (GA) also showed increased inflammasome signaling. Indeed, NLRP3 and IL-18 mRNA levels were significantly higher than those in normal eyes ($p < 0.05$).

The findings suggest inflammasome activation in RPE cells plays a destructive role in dry AMD, whereby *Alu* RNA triggers the NLRP3 inflammasome to increase the activity of IL-18 and MYD88, which in turn leads to RPE degeneration and GA.

“It is reasonable to foresee development of MyD88 inhibitors for prevention or treatment of GA,” the authors concluded in their paper in *Cell*.



Wet and dry issue

Moving forward, the Trinity team will develop an IL-18 therapy to enhance inflammasome activation in wet AMD, whereas the Kentucky team will develop MYD88 inhibitors to block inflammasome activation in dry AMD.

“Our therapeutic strategy is based on the introduction of pro-IL-18 into the retina using adeno-associated viruses (AAVs),” Trinity co-lead author Doyle told *SciBX*. Using pro-IL-18, an inactive precursor form of IL-18, “is very important from a safety point of view” because it will limit the activity of the proinflammatory cytokine to only when and where it is needed.

“Our AAV approach would be essentially self-regulating,” added co-lead author Campbell. “Pro-IL-18 is converted to its active form only in the presence of the processing enzyme caspase-1, which is expressed in the retina when there is a pathological insult such as excessive drusen prior to CNV development.”

But Ambati was skeptical about the prospects for any IL-18-based therapy. “Even if IL-18 suppresses VEGF levels and is antiangiogenic, it would probably not be a viable therapeutic strategy in any stage of AMD, given that our *Cell* paper shows activation of the inflammasome and production of IL-18 is toxic to the retinal pigment epithelium.”

Campbell countered that the findings of the *Cell* paper apply solely “to end-stage dry AMD, not wet AMD, which is what our paper addresses. We are well aware we are dealing with a proinflammatory cytokine here. Also, it must be stressed that exceedingly small amounts of IL-18 are sufficient to have a biochemical effect. We know the challenge moving forward will be determining a level of IL-18 expression that is not damaging to the microenvironment of the retina and yet can keep VEGF levels under control.”

Ambati suggested the Trinity group “make a vigorous evaluation of inflammasome and IL-18 activation levels in the eyes of wet AMD patients before pursuing therapeutic strategies along those lines.”

It would also be useful “to see if supplying exogenous IL-18 can suppress CNV in an animal model of AMD,” said Ryo Kubota, president, chairman and CEO of **Acucela Inc.**

Acucela’s ACU-4429, an oral small molecule visual cycle modulator, is in Phase IIa testing to treat dry AMD. The compound “affects a process upstream of drusen formation and prevents its accumulation,” said Kubota.

Campbell said, “In the preclinical workup, we are testing both the AAV approach as well as delivering recombinant IL-18 intravitreally. In addition, we are exploring methods of systemic administration of IL-18 or compounds that could induce expression of IL-18 locally in the eye.”

At the moment, Campbell and colleagues are analyzing IL-18 and VEGF levels “not only in the eyes of deceased AMD patients, but we are

also trying to analyze the cytokines in vitreous and aqueous samples from living patients with varying stages of AMD,” he said.

Meanwhile, iVeena Pharmaceuticals, a biotech cofounded by Ambati in 2006, has exclusively licensed the *Cell* findings from the University of Kentucky. “The company’s plan is to develop intraocular delivery of a MYD88 inhibitor to treat geographic atrophy,” said Ambati. “We will pursue that goal along three parallel lines: small molecule screens, siRNA-based therapeutics and peptide-based therapeutics.”

Bruce Ksander, assistant professor of ophthalmology at **Harvard Medical School**, said, “It is unclear to what extent dysfunction of Dicer and the accumulation of *Alu* RNA occurs in patients with geographic atrophy.”

Ambati replied that “out of the few dozen eyes we’ve studied from geographic atrophy patients, all have had elevated *Alu* RNA levels. So the phenomenon appears uniform” across the patient population.

Ksander and Patricia D’Amore, professor of ophthalmology and pathology at Harvard Medical School, have found that disruption of lysosomes in RPE cells triggers activation of the NLRP3 inflammasome. Those data have been submitted to an undisclosed peer-reviewed journal.

Ksander also is a scientist at the Schepens Eye Research Institute. D’Amore is co-director of research and a senior scientist at the Institute.

The *Nature Medicine* findings are patented and available for licensing from Trinity College.

Fulmer, T. *SciBX* 5(20); doi:10.1038/scibx.2012.511
Published online May 17, 2012

REFERENCES

- Doyle, S.L. *et al. Nat. Med.*; published online April 8, 2012; doi:10.1038/nm.2717
Contact: Matthew Campbell, Trinity College Dublin, Dublin, Ireland
e-mail: matthew.campbell@tcd.ie
- Tarallo, V. *et al. Cell*; published online April 26, 2012; doi:10.1016/j.cell.2012.03.036
Contact: Jayakrishna Ambati, University of Kentucky, Lexington, Ky.
e-mail: jamba2@email.uky.edu
- Henao-Mejia, J. *et al. Nat. Immunol.* **13**, 321–324 (2012)
- Zitvogel, L. *et al. Nat. Immunol.* **13**, 343–351 (2012)
- Duwell, P. *et al. Nature* **464**, 1357–1361 (2010)
- Masters, S.L. *et al. Nat. Immunol.* **11**, 897–904 (2010)
- Martinon, F. *et al. Nature* **440**, 237–241 (2006)
- Iyer, S.S. *et al. Proc. Natl. Acad. Sci. USA* **106**, 20388–20393 (2009)
- Kaneko, H. *et al. Nature* **471**, 325–330 (2011)

COMPANIES AND INSTITUTIONS MENTIONED

Acucela Inc., Seattle, Wash.
Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, Ohio
Harvard Medical School, Boston, Mass.
iVeena Pharmaceuticals Inc., Salt Lake City, Utah
Trinity Biomedical Sciences Institute, Dublin, Ireland
Trinity College Dublin, Dublin, Ireland
University of Kentucky, Lexington, Ky.

Paradoxical P2X7

By Tracey Baas, Senior Editor

Italian researchers have shown *in vivo* that inhibiting the P2X7 receptor, rather than agonizing it as previously thought, can treat cancer.¹ The findings could open up a new disease area for companies developing P2X7 antagonists to treat pain, multiple sclerosis, inflammatory bowel disease and rheumatoid arthritis.

P2X and P2Y receptors collectively are responsible for mediating cellular responses to extracellular ATP. Multiple groups have identified five P2Y and two P2X receptors in a variety of cancers.² The teams have shown that growth of the malignant cells were kept in check by ATP agonizing purinergic receptor P2Y G protein-coupled 1 (P2RY1; P2Y1) and P2Y2 (P2RY2) to slow cell proliferation and purinergic receptor P2X ligand-gated ion channel 5 (P2RX5; P2X5) and P2Y11 (P2RY11) to inhibit cell differentiation. In the same way, growth was checked by ATP agonizing P2X7 (P2RX7) to induce cell death.

However, two studies published in 2005 and 2009 led by Francesco Di Virgilio showed that P2X7-overexpressing cell lines were resistant to chemically triggered apoptosis and had the hallmarks of tumor transformation, including increased mitochondrial potential, synthesis of ATP and serum-free proliferation.^{3,4} Di Virgilio is a professor of clinical pathology and chairman of the Department of Experimental and Diagnostic Medicine at the **University of Ferrara**.

Inhibiting P2X7 blocked all of those hallmarks. Thus, the Italian group hypothesized that the receptor might have roles in both cell survival and cell death in cancer. Cell survival would be reliant on conditions of normal physiological levels of extracellular ATP, whereas cell death would be reliant on conditions of high pharmacological levels of ATP.

Now, Di Virgilio's group has tested this hypothesis *in vivo*. Immunodeficient mice inoculated with P2X7-overexpressing human cells generated tumors much faster than mice given human cells with normal P2X7 expression. In immunocompetent mice, inoculation with murine colon carcinoma cells that had high P2X7 expression led to much faster tumor formation than inoculation with carcinoma cells that had low P2X7 expression.

In both models, cells with high levels of P2X7 showed greater expression of VEGF and vascularization than cells that had normal levels of P2X7.

In mice inoculated with mouse melanoma or human neuroblastoma cells, P2X7 blockade with the selective P2X7 antagonist AZ10606120 or P2X7 knockdown with shRNA decreased tumor growth compared with no treatment or normal P2X7 expression, respectively.

The results were published in *Cancer Research*.

According to Di Virgilio, P2X7 antagonists have not been extensively studied in cancer because the target "is better known as a cytotoxic rather than a growth-promoting receptor. Thus, people believe that P2X7 agonists should be used to treat cancer, rather than antagonists."

"Because P2X7 receptors are widely expressed in different tissues, P2X7 antagonists will need to be shown to be selective for tumor cells with little effect on P2X7-mediated activities in other tissues."

—Geoffrey Burnstock,
*University College London
Medical School*

"I would be interested to see further animal work from Di Virgilio's team that would provide the kinetics to show if the P2X7 antagonists reach all parts of the tumors without inducing systemic toxicity or inflammation. They might need to develop special antagonists targeted at the tumor," said Pieter Dagnelie, associate professor of nutritional epidemiology at **Maastricht University**.

Geoffrey Burnstock, emeritus professor and president of the Autonomic Neuroscience Centre of the **University College London Medical School**, agreed. "Because P2X7 receptors are widely expressed in different tissues, P2X7 antagonists will need to be shown to be selective for tumor cells with little effect on P2X7-mediated activities in other tissues. Also, there are many human P2X7 polymorphisms that might not all respond to an antagonist."

"There is a strong need to go beyond overexpression studies and immunostaining of P2X7 in tumor samples and rather address dependence of cancers on P2X7 for initiation, maintenance and metastasis," said Joanna Hergovich Lisztwan, research leader of oncology at **Evotec AG**. "There needs to be a deeper understanding of how the exact mechanisms

involved in P2X7's regulation of mitochondrial metabolism would lend further argument to targeting this ion channel in cancer, or alternatively reveal new targets involved in the pathway."

Evotec's EVT 401, an oral small molecule P2X7 receptor antagonist, is in Phase I testing. Preclinical studies are ongoing to assess its potential in a number of indications.

Immune issues

In addition to sorting out the distribution of P2X7 receptors, another question is whether blocking the target will modulate how the immune system interacts with tumors.

For example, George Dubyak, professor of physiology and biophysics at **Case Western Reserve University**, said antagonizing the receptor "could also suppress P2X7 signaling in dendritic cells and macrophages that is essential for the antitumor immune responses elicited by several chemotherapeutic agents."

When chemotherapeutics destroy tumor cells, high levels of ATP are released from the tumor interstitial space. The ATP then acts on dendritic cell-specific P2X7 to trigger the inflammasome and production of IL-1 β and IL-18, resulting in immune system-mediated eradication of cancer cells.⁵

Di Virgilio countered that his team showed that "P2X7 antagonists caused tumor regression in immunocompetent mice. This is a first step toward showing that the immunological component might still be effective. But future studies in mice will need to include extended time courses while looking closely at immune-response components."

John Lust and Kathleen Donovan, both in the Division of Hematology at the **Mayo Clinic**, said P2X7 inhibitors might have additional upside by blocking chemotherapy-induced inflammation. "My laboratory has previously shown in samples from myeloma patients that P2X7-specific inhibitors developed by **Pfizer Inc.** can block ATP-induced IL-1 β release and subsequent IL-6 production, particularly in chemotherapy-treated

patients. IL-6 is a central growth factor for myeloma cells. We believe our results suggest a way to inhibit chemotherapy-induced inflammation that may be contributing to relapse seen with this disease.”

Lust's team tested four P2X7 antagonists from Pfizer. Lust did not disclose any further details regarding the specific molecules or his collaboration with Pfizer.

P2X7 antagonists in development include **GlaxoSmithKline plc's** GSK1482160, a purinergic ATP receptor antagonist that targets P2X7. The molecule is in Phase I testing to treat pain. The pharma declined to comment for this story.

Affectis Pharmaceuticals AG's AFC-5128, a brain-penetrant P2X7 antagonist, is in preclinical development to treat multiple sclerosis (MS) and neuropathic pain. The company expects to submit an IND within 12 months.

Affectis' CBO Luc St-Onge is looking forward to seeing results from the new paper extended to other mouse models of cancer, especially brain cancer.

Next steps from Di Virgilio's team include more extensive animal studies with P2X7 antagonists that have already gone through Phase I and II testing for other indications.

The work is not patented and is available for licensing.

Baas, T. *SciBX* 5(20); doi:10.1038/scibx.2012.512

Published online May 17, 2012

REFERENCES

1. Adinolfi, E. *et al. Cancer Res.*; published online April 13, 2012; doi:10.1158/0008-5472.CAN-11-1947
Contact: Francesco Di Virgilio, University of Ferrara, Ferrara, Italy
e-mail: fdv@unife.it
2. White, N. & Burnstock, G. *Trends Pharmacol. Sci.* 27, 211–217 (2006)
3. Adinolfi, E. *et al. Mol. Biol. Cell* 16, 3270–3272 (2005)
4. Adinolfi, E. *et al. J. Biol. Chem.* 284, 10120–10128 (2009)
5. Zitvogel, L. *et al. Nat. Immunol.* 13, 343–351 (2012)

COMPANIES AND INSTITUTIONS MENTIONED

Affectis Pharmaceuticals AG, Martinsried, Germany

Case Western Reserve University, Cleveland, Ohio

Evotec AG (Xetra:EVT), Hamburg, Germany

GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.

Maastricht University, Maastricht, the Netherlands

Mayo Clinic, Rochester, Minn.

Pfizer Inc. (NYSE:PFE), New York, N.Y.

University College London Medical School, London, U.K.

University of Ferrara, Ferrara, Italy

SciBX: Science–Business eXchange

Kick-start your knowledge management—and leave your competitors behind...

Can you afford not to subscribe?

Visit scibx.com for details on how to subscribe to SciBX

K-Ras in cancer metabolism

By Lauren Martz, Staff Writer

Researchers from the **Dana-Farber Cancer Institute** have identified two glucose metabolism pathways that are activated by the *K-Ras* oncogene in pancreatic tumors.¹ Based on the findings, it may be possible to target proteins in the pathways to block proliferation of *K-Ras*-driven cancers.

Tumors typically grow and proliferate by dramatically increasing their rate of glycolysis compared with that in normal cells. Cancers rely on the process, called the Warburg effect, because it supplies energy and intermediates to sustain tumor growth. Thus, blocking glycolysis and potentially other metabolic pathways in tumors could cut off the malignancy's energy and nutrient supply.

Indeed, potential therapeutic targets in cancer metabolism pathways include pyruvate kinase M2 isoenzyme (PKM2) and isocitrate dehydrogenase 1 (IDH1).^{2,3} Several oncogenes have also been implicated in the activation of cancer metabolism pathways, including *PTEN* (*MMAC1*; *TEP1*), *c-Myc* (*MYC*), and *phosphoinositide 3-kinase* (*PI3K*) and *protein kinase B* (*PKB*; *PKBA*; *AKT*; *AKT1*).⁴⁻⁶

Now, Alec Kimmelman and colleagues at Dana-Farber have shown that the *K-Ras* oncogene upregulates two glucose metabolism pathways in pancreatic tumors. The team also identified enzymes in each of the pathways that could become new therapeutic targets for pancreatic cancer.

K-Ras mutations are best known as drivers of resistance to epidermal growth factor receptor (EGFR) inhibitors. Activating mutations in *K-Ras* are found in more than 90% of patients with pancreatic cancer. Although the gene is a driver for cancer initiation and progression, there are no disclosed small molecule inhibitors selective for mutant *K-Ras* in development because attempts to target the complex biology and interactions of the mutant form of the protein have been unsuccessful.

Prior work had shown that mutant *K-Ras* drives tumor growth in part by hijacking metabolic pathways in tumors. Those results suggested it might be possible to block the effects of *K-Ras* by going downstream and targeting proteins in a protumorigenic metabolic pathway. The challenge was identifying which metabolic pathways mutant *K-Ras* hijacked.

To find those pathways, the Dana-Farber team designed a mouse model of pancreatic ductal adenocarcinoma (PDAC) that expressed *K-ras* in the pancreas only in the presence of doxycycline. To further increase the number of pancreatic lesions that progressed to PDAC, the team also knocked out the tumor suppressor p53.

In the mice, doxycycline withdrawal caused tumor regression through increased apoptosis and decreased cancer cell proliferation compared with continued doxycycline exposure, confirming that maintenance of pancreatic tumors required *K-ras*.

Genetic and metabolic studies showed glycolytic metabolites and

enzymes were downregulated in the absence of *K-Ras*. Downregulated enzymes included glutamine-fructose-6-phosphate transaminase 1 (Gfpt1), ribose 5-phosphate isomerase A (Rpia) and ribulose-5-phosphate-3-epimerase (Rpe).

Finally, the team showed that treatment of the *K-ras*-mutant tumors with a MEK inhibitor downregulated the same set of metabolism genes. However, treatment with inhibitors of mammalian target of rapamycin (mTOR; FRAP; RAFT1) or the PI3K and AKT pathway did not reduce gene expression.

Those findings suggest the MEK pathway downstream of *K-Ras* is the relevant mechanism that promotes cancer metabolism in pancreatic tumors, whereas other pathways such as the PI3K and mTOR pathway do not affect cancer metabolism.

Kimmelman is an assistant professor of radiation oncology at Dana-Farber and **Harvard Medical School**. The paper also included researchers from **Beth Israel Deaconess Medical Center, Massachusetts General Hospital** and **The University of Texas MD Anderson Cancer Center**.

Results were published in *Cell*.

"The fact that many oncogenic or tumor suppressor pathways have an impact on cell metabolism has been one of the key factors underpinning the recent resurgence in cancer metabolism research," said Neil Jones, senior principal target validation scientist at **Cancer Research UK's Cancer Research Technology Ltd.** commercial arm.

Cancer Research Technology and **AstraZeneca plc** are identifying cancer metabolism targets and developing therapeutics against them under a three-year deal.

"The research area of cancer metabolism is undergoing a renaissance driven by a greater understanding of how genetic drivers reprogram metabolic pathways," added Patrick O'Connor, VP and head of oncology at **Ruga**

Corp. The company has multiple preclinical programs focused on tumor-selective adaptive responses including tumor metabolism.

"Because it is difficult to inhibit the *Ras* oncogene directly, this paper provides a number of downstream mediators that could become alternative therapeutic targets for *Ras*-driven tumors. The goal is to engineer potent and selective inhibitors impacting various points in the pathways uncovered in this manuscript as a step toward therapeutic intervention in the clinic," said O'Connor.

Based on the new findings, GFPT1 "could be a potential target, as glycosylation is an intriguing area of cancer research, with this post-translational modification playing a fundamental role in many tumor-related responses including proliferation, invasion, immune response and angiogenesis," added Jones.

O'Connor said the findings should also encourage "further preclinical investigation of MEK inhibition with inhibitors of glucose uptake or inhibitors of glucose utilization through the various pathways identified."

Next steps

Kimmelman told *SciBX* his team is performing further mechanistic studies on the key pathways elucidated in the paper and is designing

"The research area of cancer metabolism is undergoing a renaissance driven by understanding the genetic drivers and how they turn on and drive metabolic pathways."

—Patrick O'Connor, Ruga Corp.

inhibitors of the newly identified targets.

Jones said a key area of investigation “would be to evaluate the role of different *K-Ras* mutations in tumor metabolism. There is evidence that different *K-Ras* mutants utilize different effector pathways that could give rise to an alternative metabolic phenotype.”

“It will also be important to evaluate the expression and significance of some of the identified targets in clinical samples of *K-Ras*-dependent prostate cancers to see if the identified metabolism enzyme signature translates into clinical samples,” said Jones.

He said it also will be necessary to study “potential bypass mechanisms of alternative metabolic pathways and any toxicity implication in rapidly proliferating normal tissues.”

Finally, because the potential cancer metabolism targets described in the paper were identified primarily through genetic studies, a key next step will be to determine whether selective and potent pharmacologic inhibitors of these reprogrammed pathways can safely reproduce the same results, said O’Connor.

Kimmelman said Dana-Farber has filed a patent application covering the findings and the new targets. The IP is available for licensing.

Martz, L. *SciBX* 5(20); doi:10.1038/scibx.2012.513
Published online May 17, 2012

REFERENCES

1. Ying, H. *et al. Cell*; published online April 27, 2012; doi:10.1016/j.cell.2012.01.058
Contact: Ronald A. DePinho, The University of Texas MD Anderson Cancer Center, Houston, Texas
e-mail: rdepinho@mdanderson.org
Contact: Alec C. Kimmelman, Dana-Farber Cancer Institute, Boston, Mass.
e-mail: alec_kimmelman@dfci.harvard.edu
2. Christofk, H.R. *et al. Nature* **452**, 230–233 (2008)
3. Thompson, C.B. *N. Eng. J. Med.* **360**, 813–813 (2009)
4. Shim, H. *et al. Proc. Natl. Acad. Sci. USA* **94**, 6658–6663 (1997)
5. Doe, M.R. *et al. Cancer Res.* **72**, 949–957 (2012)
6. Govindarajan, B. *et al. J. Clin. Invest.* **117**, 719–729 (2007)

COMPANIES AND INSTITUTIONS MENTIONED

AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Beth Israel Deaconess Medical Center, Boston, Mass.
Cancer Research Technology Ltd., London, U.K.
Cancer Research UK, London, U.K.
Dana-Farber Cancer Institute, Boston, Mass.
Harvard Medical School, Boston, Mass.
Massachusetts General Hospital, Boston, Mass.
Ruga Corp., Palo Alto, Calif.
The University of Texas MD Anderson Cancer Center, Houston, Texas

Can You Afford Not to Read SciBX?

According to MEDLINE®, the U.S. National Library of Medicine’s® premier bibliographic database of articles in life sciences, over 775,000 articles were added to the database in 2009 alone—an average of almost 15,000 new articles every week.

Can you afford to miss investment opportunities?

Can you afford to miss emerging competition?

SciBX is the single source for scientific context, commercial impact and the critical next steps.

Visit scibx.com for details on how to subscribe to SciBX

SciBX: Science–Business eXchange

PROTAC the protein

By Kai-Jye Lou, Staff Writer

GlaxoSmithKline plc and Yale University researchers have announced a collaboration to develop a platform that selectively tags disease-associated proteins with an E3 ubiquitin ligase ligand, thus targeting them to a cell's protein degradation machinery. GSK hopes the platform could be a cornerstone for a new Discovery Performance Unit at the pharma, and the partners are aiming to have proof-of-principle results in cell culture by year end.

The platform is based on a chemistry technology called proteolysis-targeting chimeric molecules, or PROTACs, which was first reported by Craig Crews at Yale and colleagues at the California Institute of Technology in 2001.¹ In a pair of recent papers, Crews' team brought the technology closer to therapeutic application by developing small molecule PROTACs.^{2,3}

In contrast to typical small molecule inhibitors that block a protein's activity by binding its active site, PROTACs inhibit activity by promoting a protein's degradation via the ubiquitin-proteasome system. The heterodimeric PROTACs do this by forming noncovalent links between the target protein and an E3 ubiquitin ligase, which then marks the protein for degradation by ubiquitinating it.

Crews said PROTACs could make it possible to target intracellular proteins, such as transcription factors and the GTPase K-Ras, that historically have been undruggable with conventional small molecules.

"The structure of the target protein isn't even required, as one just needs to screen molecular libraries for compounds that bind to the target. This liberates one from the limitation of having to develop molecules that target an active site," said Crews, who is a professor of chemistry, pharmacology, and molecular, cellular and developmental biology at Yale.

He said any location on the target protein in which a molecule binds could be exploited by a PROTAC.

The challenge now is to design PROTACs that are more drug-like, which is where GSK comes in, said Crews.

"Our earlier PROTAC molecules were peptidic and large, which could make them difficult to synthesize on a large scale, unstable and difficult to get into cells," he told *SciBX*. "But we have recently come up with a small molecule E3 ligand, which we are now using as a starting point in the design of molecules that could be linked with targeting moieties against various proteins."

According to Crews, the move from large and peptidic to small molecule was the key transition that caught GSK's attention. He said GSK reached out to the group last year to discuss the potential of developing PROTACs into medicines.

"Some of the findings from the Yale group last year led us to believe that we could make small molecule medicines with the approach," said Kris Famm, who heads GSK's protein degradation effort. "Small molecules are entities that we have many decades of experience with, and we will be able to use our in-house medicinal chemistry expertise and capabilities to generate such PROTACs with more drug-like properties."

Famm said the idea to explore the therapeutic potential of PROTACs followed a call for proposals for new GSK Discovery Performance Units last fall. GSK Discovery Performance Units are small biotech-like groups that focus on specific R&D projects. These units submit project proposals and seek funding from the pharma's Discovery Investment Board.

"I reached out to the Crews lab to explore the potential to develop these tools into medicines and to see whether this could be a cornerstone for a new GSK Discovery Performance Unit," Famm told *SciBX*. "This coincided with progress in the Crews lab on making small molecule E3 ubiquitin ligase-binding moieties."

Running interference

Crews thinks the key competitor for PROTACs is RNAi, which also offers the ability to target proteins regardless of class.

He said PROTACs could have an advantage over RNAi because the latter operates at the level of RNA and thus may have difficulty distinguishing between different conformations of the same protein.

"Because PROTACs work at the level of proteins, they could, for example, be designed to specifically target an oncogenic form of the protein for degradation without also targeting the normal form expressed in healthy cells," Crews told *SciBX*.

He added that technical issues make it difficult to deliver and titrate the effect of RNAi-based molecules.

In addition, Crews noted that the mechanism of PROTACs also could give them an edge over existing drugs that are rapidly metabolized and cleared from the body.

"Instead of having to constantly take a particular drug to achieve the necessary level of inhibition for a therapeutic effect, PROTACs reduce the levels of the protein itself so that even when the PROTAC has been cleared, the body will still need to resynthesize the targeted protein to levels that could be harmful," he said.

Famm said PROTACs could have applications across a range of indications in which a protein is expressed at higher levels in diseased tissue than healthy tissue. GSK is not disclosing the specific disease areas and proteins that it plans to target at this time.

"We've established a set of proof-of-principle proteins that we would like to target, and the goal by the end of the year is to show in cell culture that PROTACs could be used to knock down these target proteins," Famm told *SciBX*. "We will also want to show that the technology is generally applicable and prove that we could generate a broad range of PROTACs that are selective, cell permeable and able to exert their intracellular effect. If preclinical proof of principle is achieved for the PROTAC platform, GSK hopes to lead the way in drugging a range of validated disease targets that haven't been possible to effectively target with traditional inhibitors."

Under the collaboration, the GSK Discovery Investment Board and the university will jointly contribute the resources required for the proof-of-principle studies. Yale will be eligible for undisclosed milestones and royalties on each PROTAC that is selected for further development.

"We have recently come up with a small molecule E3 ligand, which we are now using as a starting point in the design of molecules that could be linked with targeting moieties against various proteins."

— Craig Crews, Yale University

GSK has licensed the PROTAC technology from Yale for use for multiple targets. The university retains control of IP related to the small molecule E3 ubiquitin ligase-binding fragment of the PROTACs and its general use. GSK will have exclusive rights to the IP covering whole PROTACs developed under the collaboration.

Lou, K.-J. *SciBX* 5(20); doi:10.1038/scibx.2012.514
Published online May 17, 2012

REFERENCES

1. Sakamoto, K.M. *et al. Proc. Natl. Acad. Sci. USA* **98**, 8554–8559 (2001)
2. Buckley, D.L. *et al. J. Am. Chem. Soc.* **134**, 4465–4468 (2012)
3. Neklesa, T.K. *et al. Nat. Chem. Biol.* **7**, 538–543 (2011)

COMPANIES AND INSTITUTIONS MENTIONED

California Institute of Technology, Pasadena, Calif.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Yale University, New Haven, Conn.



The Scientific Acumen of Nature Publishing Group
plus
The Business Intelligence of BioCentury Publications, Inc.
in a single publication

Can you afford not to subscribe?
Visit **scibx.com** for details on how to subscribe to SciBX

BioCentury

nature publishing group 

This week in therapeutics

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

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

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer				
Breast cancer	Neutrophil elastase (NE; ELA-2); cyclin E (CCNE)	<i>In vitro</i> studies suggest a vaccine based on a peptide that NE cleaves from CCNE could help treat breast cancer. In breast cancer cell lines, NE cleaved full-length CCNE, which led to an increase in cell surface expression of CCNE ₁₄₄₋₁₅₂ compared with that seen using a control protease. In breast cancer cell lines pretreated with NE, compared with non-pretreated cell lines, primary CCNE ₁₄₄₋₁₅₂ -specific T cells increased cancer cell death. Planned work includes a Phase I trial of a CCNE ₁₄₄₋₁₅₂ vaccine to treat breast cancer. SciBX 5(20); doi:10.1038/scibx.2012.515 Published online May 17, 2012	Patented by The University of Texas MD Anderson Cancer Center; available for licensing	Mittendorf, E.A. <i>et al. Cancer Res.</i> ; published online May 7, 2012; doi:10.1158/0008-5472.CAN-11-4135 Contact: Elizabeth A. Mittendorf, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: eamitten@mdanderson.org Contact: Jeffrey J. Mollidrem, same affiliation as above e-mail: jmollidre@mdanderson.org
Cancer	Phosphoinositide 3-kinase-β (PI3Kβ); PTEN (MMAC1; TEP1)	A study in mice identified PI3Kβ-selective inhibitors that could help treat PTEN-deficient cancers. In a mouse xenograft model of PTEN-deficient prostate cancer, benzimidazole-pyrimidone-based and benzoxazole-pyrimidone-based PI3Kβ inhibitors decreased tumor growth compared with vehicle. Next steps could include testing the inhibitors in other models of PTEN-deficient cancers. SciBX 5(20); doi:10.1038/scibx.2012.516 Published online May 17, 2012	Patent and licensing status unavailable	Certal, V. <i>et al. J. Med. Chem.</i> ; published online April 23, 2012; doi:10.1021/jm300241b Contact: Frank Halley, Sanofi Research & Development, Vitry-sur-Seine, France e-mail: frank.halley@sanofi.com
Pancreatic cancer	Glutamine-fructose-6-phosphate transaminase 1 (GFPT1); K-Ras; MEK; ribose 5-phosphate isomerase A (RPIA)	<i>In vitro</i> studies identified targets involved in pancreatic cancer metabolism that could help treat K-Ras-driven cancers. In a mouse model of K-Ras-driven pancreatic ductal adenocarcinoma, removal of K-Ras expression caused tumor regression and downregulation of genes including <i>Gpft1</i> and <i>Rpia</i> , which regulate cellular pathways related to glucose metabolism. In the cancer cells, inhibiting MEK reduced expression of the same set of cancer metabolism genes as removing K-Ras expression. Next steps include conducting mechanistic studies of the pathways and designing inhibitors. At least 10 companies have MEK inhibitors in clinical and preclinical testing to treat various cancers (<i>see K-Ras in cancer metabolism, page 7</i>). SciBX 5(20); doi:10.1038/scibx.2012.517 Published online May 17, 2012	Patent application filed by the Dana-Farber Cancer Institute; available for licensing	Ying, H. <i>et al. Cell</i> ; published online April 27, 2012; doi:10.1016/j.cell.2012.01.058 Contact: Ronald A. DePinho, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: rdepinho@mdanderson.org Contact: Alec C. Kimmelman, Dana-Farber Cancer Institute, Boston, Mass. e-mail: alec_kimmelman@dfci.harvard.edu
Cardiovascular disease				
Arrhythmia	Phosphoinositide 3-kinase (PI3K)	<i>In vitro</i> and mouse studies suggest some doses of PI3K inhibitors may induce long QT syndrome when used to treat cancer. In cultured canine myocytes, the small molecule PI3K inhibitors BEZ235 and PI-103 produced dose-dependent prolongation of action potential, whereas vehicle did not. In mice, cardiac-specific knockout of the Pi3kα isoform led to action potential prolongation, whereas knockout of Pi3kβ had minimal effects on the action potential. Next steps include developing a method to reduce the risk of long QT syndrome when dosing PI3K inhibitors. BEZ235, a dual inhibitor of PI3Kα and mammalian target of rapamycin (mTOR; FRAP; RAFT1) from Novartis AG, is in Phase I/II testing to treat solid cancers. At least 27 other companies have compounds that inhibit PI3K in clinical and preclinical testing to treat cancer. PI-103 is a research reagent. SciBX 5(20); doi:10.1038/scibx.2012.518 Published online May 17, 2012	Unpatented; licensing status not applicable	Lu, Z. <i>et al. Sci. Transl. Med.</i> ; published online April 25, 2012; doi:10.1126/scitranslmed.3003623 Contact: Ira S. Cohen, State University of New York at Stony Brook, Stony Brook, N.Y. e-mail: ira.cohen@stonybrook.edu Contact: Richard Z. Lin, same affiliation as above e-mail: richard.lin@stonybrook.edu

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cardiomyopathy	VEGF receptor 1 (FLT1; VEGFR-1)	Human and mouse studies suggest inhibiting soluble FLT1 (sFLT1) could help prevent or treat peripartum cardiomyopathy (PPCM) in women with preeclampsia, a known risk factor for PPCM. In postpartum women with preeclampsia and PPCM, sFLT1 levels were up to 10-fold higher than those in postpartum women who had neither condition. In prepartum women with preeclampsia, elevated sFLT1 levels correlated with cardiac dysfunction. In normal pregnant and nonpregnant mice, mouse sFLT1 decreased cardiac function compared with vehicle. Ongoing work includes identifying small molecule inhibitors of sFLT1 secretion. SciBX 5(20); doi:10.1038/scibx.2012.519 Published online May 17, 2012	Unpatented; available for partnering	Patten, I.S. <i>et al. Nature</i> ; published online May 9, 2012; doi:10.1038/nature11040 Contact: Zoltan Arany, Harvard Medical School, Boston, Mass. e-mail: zarany@bidmc.harvard.edu Contact: Denise Hilfiker-Kleiner, Hannover Medical School, Hannover, Germany e-mail: hilfiker.denise@mh-hannover.de
Cardiovascular disease	Perilipin 5 (PLIN5)	Mouse studies suggest PLIN5 could help protect against fatty acid–induced oxidative stress and cardiac toxicity. <i>Plin5</i> knockout mice had greater production of reactive oxygen species (ROS) in the heart and had faster age-related decline of cardiac function than wild-type controls. Next steps include studying the role of PLIN5 in human cardiac diseases. SciBX 5(20); doi:10.1038/scibx.2012.520 Published online May 17, 2012	Unpatented; unavailable for licensing	Kuramoto, K. <i>et al. J. Biol. Chem.</i> ; published online April 24, 2012; doi:10.1074/jbc.M111.328708 Contact: Takashi Osumi, University of Hyogo, Hyogo, Japan e-mail: osumi@sci.u-hyogo.ac.jp
Heart failure	Toll-like receptor 9 (TLR9)	Mouse studies suggest inhibiting TLR9 could help prevent heart failure caused by inflammation. In mice subjected to cardiac pressure overload, <i>Thr9</i> deficiency increased survival and decreased loss of cardiac function and pulmonary congestion compared with normal <i>Thr9</i> expression. Next steps could include testing TLR9 inhibitors in the mouse model. At least 10 companies have TLR9 inhibitors in preclinical or clinical development for various indications, including inflammation. SciBX 5(20); doi:10.1038/scibx.2012.521 Published online May 17, 2012	Unpatented; licensing status not applicable	Oka, T. <i>et al. Nature</i> ; published online April 25, 2012; doi:10.1038/nature10992 Contact: Kinya Otsu, King's College London, London, U.K. e-mail: kinya.otsu@kcl.ac.uk
Endocrine/metabolic disease				
Diabetes; dyslipidemia	Not applicable	Rodent studies suggest hydroxyflavone analogs could help treat type 2 diabetes and dyslipidemia. In mouse and rat models of diabetes, the lead analog decreased plasma glucose and insulin levels by 55% and 53%, respectively, and increased glucose tolerance by 39% compared with vehicle. In the mouse models, the lead compound lowered plasma triglyceride levels and total cholesterol by 31% and 27%, respectively, compared with vehicle. Future studies could include lead optimization and identification of the molecular target of the analogs. SciBX 5(20); doi:10.1038/scibx.2012.522 Published online May 17, 2012	Patent and licensing status unavailable	Verma, A.K. <i>et al. J. Med. Chem.</i> ; published online April 23, 2012; doi:10.1021/jm201107g Contact: Ram Pratap, Central Drug Research Institute, Lucknow, India e-mail: r_pratap@cdri.res.in
Infectious disease				
Bacterial infection	DNA repair	<i>In vitro</i> studies identified a common mechanism of antibiotic-induced cell death that could aid the design of antibiotic adjuvants. In cultured <i>Escherichia coli</i> cells, the antibiotics ampicillin, norfloxacin and kanamycin, which act via three different pathways, all induced cell death in part by oxidizing guanine to 8-oxo-deoxyguanine (8-oxo-dG) to induce DNA double-stranded breaks. Next steps could include testing inhibitors of DNA double-stranded break repair as antibiotic adjuvants. SciBX 5(20); doi:10.1038/scibx.2012.523 Published online May 17, 2012	Patent and licensing status unavailable	Foti, J.J. <i>et al. Science</i> ; published online April 19, 2012; doi:10.1126/science.1219192 Contact: Graham C. Walker, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: gwalker@mit.edu

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Influenza virus	Influenza A virus PB1-F2 protein (PB1-F2)	<i>In vitro</i> and mouse studies identified a PB1-F2 inhibitor that could help treat influenza. <i>In vitro</i> , the compound inhibited the growth of influenza virus, including H1N1 pandemic and avian H5N1 strains, but not influenza virus containing a PB1-F2 mutation. In mice, the inhibitor partially protected against lethal influenza challenge and increased survival compared with saline control. Next steps include confirming that PB1-F2 is the direct target of the inhibitor. SciBX 5(20); doi:10.1038/scibx.2012.524 Published online May 17, 2012	Patent application filed; available for licensing	Ortigoza, M.B. <i>et al. PLoS Pathog.</i> ; published online April 26, 2012; doi:10.1371/journal.ppat.1002668 Contact: Megan L. Shaw, Mount Sinai School of Medicine, New York, N.Y. e-mail: megan.shaw@mssm.edu
Inflammation				
Inflammation	Phosphoinositide 3-kinase- γ (PI3K γ)	<i>In vitro</i> and mouse studies identified a sulfamoylphenyl pyrazine PI3K γ inhibitor that could help treat inflammatory diseases. In <i>in vitro</i> assays, the compound showed good selectivity for PI3K γ and inhibited its target with nanomolar IC ₅₀ values. In a mouse model of inflammation, the compound decreased both mast cell degranulation in ear skin cells and neutrophil recruitment to the peritoneal cavity compared with vehicle. Next steps could include testing the inhibitors in animal models of inflammatory and autoimmune diseases. SciBX 5(20); doi:10.1038/scibx.2012.525 Published online May 17, 2012	Patent applications filed covering findings; available for licensing	Leahy, J.W. <i>et al. J. Med. Chem.</i> ; published online May 1, 2012; doi:10.1021/jm300403a Contact: Henry W.B. Johnson, Exelixis Inc., South San Francisco, Calif. e-mail: hjohnson@exelixis.com
Neurology				
Huntington's disease (HD)	Diacylglycerol kinase- ϵ (DGKE)	Mouse, fly and cell culture studies suggest inhibiting DGKE could help treat HD. In mouse striatal neurons that express mutant Huntingtin (Htt), diacylglycerol kinase inhibitors decreased Htt toxicity compared with no treatment. In a mouse model of HD, Dgke levels were greater than those in control mice. In a fly model of HD, small hairpin RNA knockdown of <i>Dgke</i> delayed disease onset and decreased Htt toxicity and increased motor performance compared with normal <i>Dgke</i> expression. Next steps include identifying selective inhibitors of DGKE. SciBX 5(20); doi:10.1038/scibx.2012.526 Published online May 17, 2012	Unpatented; licensing status not applicable	Zhang, N. <i>et al. J. Biol. Chem.</i> ; published online April 16, 2012; doi:10.1074/jbc.M111.321661 Contact: Lisa M. Ellerby, Buck Institute for Research on Aging, Novato, Calif. e-mail: lellerby@buckinstitute.org
Pain	Bradykinin B1 receptor (BDKRB1; B1R)	An <i>in vitro</i> and mouse study identified cyclic peptide-based B1R antagonists that could help treat pain. The antagonists were created by linking known peptide inhibitors of B1R to kalata B1, an orally available cysteine-rich plant peptide with a cyclic backbone. In a mouse model of visceral pain, oral dosing of the new antagonists decreased pain compared with dosing of the linear parent peptides. Next steps include determining the bioavailability of the new inhibitors in small animals. Evotec AG has a B1R antagonist in preclinical development for pain and inflammation. SciBX 5(20); doi:10.1038/scibx.2012.527 Published online May 17, 2012	Patent application filed; available for licensing	Wong, C.T.T. <i>et al. Angew. Chem. Int. Ed.</i> ; published online April 24, 2012; doi:10.1002/anie.201200984 Contact: James P. Tam, Nanyang Technological University, Singapore, Singapore e-mail: jptam@ntu.edu.sg

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Pain	Monoacylglycerol lipase (MAGL)	<i>In vitro</i> and rodent studies identified a class of O-hexafluoroisopropyl (HFIP) carbamates that selectively inhibited MAGL and could help treat pain. In a serine hydrolase assay, the HFIP carbamates were more selective than existing O-aryl carbamates for MAGL. In mice, the new inhibitors potently blocked MAGL with no detectable cross-reactivity with fatty acid amide hydrolase (FAAH), whereas previously identified inhibitors had low levels of cross-reactivity. Next steps include preclinical safety profiling of the class of compounds. Abide Therapeutics Inc. has MAGL inhibitors in preclinical development to treat neuropathic pain.	Patent application filed covering MAGL inhibitors; licensed by Abide	Chang, J.W. <i>et al. Chem. Biol.</i> ; published online April 26, 2012; doi:10.1016/j.chembiol.2012.03.009 Contact: Benjamin F. Cravatt, The Scripps Research Institute, La Jolla, Calif. e-mail: cravatt@scripps.edu Contact: Micah J. Niphakis, same affiliation as above e-mail: mniphak@scripps.edu
Ophthalmic disease				
Age-related macular degeneration (AMD)	IL-18	Mouse studies suggest local delivery of IL-18 to the retina could help treat wet AMD. In a mouse model of wet AMD, knockout of the NLR family pyrin domain containing 3 (Nlrp3; Nalp3) inflammasome, an IL-18 activator, increased retinal damage compared with wild-type inflammasome expression. Also in the mouse models, knockout of IL-18 or an anti-IL-18 mAb increased retinal lesions compared with normal IL-18 expression or vehicle control, respectively. Next steps include developing a method for local delivery of pro-IL-18 to the retina using adeno-associated virus (AAV) vectors (<i>see Eyeing the inflammasome, page 1</i>).	Patented; available for licensing from the Trinity College Dublin research and innovation office Contact: Emily Vereker, Trinity College Dublin, Dublin, Ireland e-mail: vereeke@tcd.ie	Doyle, S.L. <i>et al. Nat. Med.</i> ; published online April 8, 2012; doi:10.1038/nm.2717 Contact: Matthew Campbell, Trinity College Dublin, Dublin, Ireland e-mail: matthew.campbell@tcd.ie
Age-related macular degeneration (AMD)	Myeloid differentiation primary response gene 88 (MYD88)	Mouse studies suggest inhibiting MYD88 in the retinal pigment epithelium (RPE) could help treat dry AMD. Geographic atrophy, an advanced form of dry AMD, can be triggered by aberrant buildup of <i>Alu</i> RNA in the RPE. In mice with excess <i>Alu</i> RNA in the RPE, knockout of <i>NLR family pyrin domain containing 3 (Nlrp3; Nalp3)</i> , <i>Myd88</i> or <i>Il-18</i> all decreased RPE degeneration compared with normal expression. Also in the mice, a peptide-based MYD88 inhibitor prevented <i>Alu</i> RNA-induced retinal damage. Next steps at iVeena Pharmaceuticals Inc., the licensee of the findings, include screening for and developing small molecule-, small interfering RNA- or peptide-based inhibitors of MYD88 (<i>see Eyeing the inflammasome, page 1</i>).	Findings covered by patent applications; licensed to iVeena	Tarallo, V. <i>et al. Cell</i> ; published online April 26, 2012; doi:10.1016/j.cell.2012.03.036 Contact: Jayakrishna Ambati, University of Kentucky, Lexington, Ky. e-mail: jamba2@email.uky.edu
Pulmonary disease				
Emphysema	Sirtuin 1 (SIRT1)	Mouse studies suggest activating SIRT1 could help treat emphysema. In mice, <i>Sirt1</i> knockout in airway epithelium decreased lung function and tolerance to exercise compared with normal <i>Sirt1</i> expression. In mice exposed to cigarette smoke or elastase, <i>Sirt1</i> overexpression or treatment with the SIRT1 activator SRT1720 decreased emphysema symptoms compared with normal <i>Sirt1</i> expression or vehicle, respectively. Next steps include IND-enabling preclinical studies. GlaxoSmithKline plc's Sirtris Pharmaceuticals Inc. subsidiary has SIRT1 activators, including SRT1720, in clinical and preclinical testing for various conditions. Elixir Pharmaceuticals Inc. has a SIRT1 activator in preclinical testing to treat obesity and diabetes.	Patent applications filed; available for licensing	Yao, H. <i>et al. J. Clin. Invest.</i> ; published online May 1, 2012; doi:10.1172/JCI60132 Contact: Irfan Rahman, University of Rochester Medical Center, Rochester, N.Y. e-mail: irfan_rahman@urmc.rochester.edu

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Renal disease				
Nephropathy	Uromodulin (UMOD; THP)	Rat studies suggest antagonizing UMOD binding to immunoglobulin light chain could be useful for treating nephropathy associated with multiple myeloma. Prior studies showed that in patients with multiple myeloma, binding of immunoglobulin light chain to UMOD in the kidney leads to nephropathy. <i>In vitro</i> , a cyclic peptide mimicking the UMOD-binding region of immunoglobulin light chain blocked the peptide–light chain interaction. In a rat model of immunoglobulin light chain–induced nephropathy, i.v. injection of this competitive peptide prevented nephropathy, whereas injection of a noncompetitive peptide had no effect. Next steps include toxicological and pharmacodynamic studies of the peptide. SciBX 5(20); doi:10.1038/scibx.2012.532 Published online May 17, 2012	Patent pending; available for licensing	Ying, W.-Z. <i>et al. J. Clin. Invest.</i> ; published online April 9, 2012; doi:10.1172/JCI46490 Contact: Paul W. Sanders, The University of Alabama at Birmingham, Birmingham, Ala. e-mail: psanders@uab.edu
Various				
Autoimmune disease; inflammation	Phosphoinositide 3-kinase- γ (PI3K γ)	<i>In vitro</i> , cell culture and mouse studies suggest highly selective inhibitors of PI3K γ could help treat inflammation and autoimmune diseases. <i>In vitro</i> screening and optimization led to the development of CZC24832, a small molecule inhibitor that is at least 100 times more selective for PI3K γ over the other PI3K isoforms. In cell culture, CZC24832 decreased differentiation of proinflammatory T helper type 17 (Th17) cells compared with vehicle. In a mouse model of collagen-induced arthritis, CZC24832 lowered bone and cartilage destruction by 53% compared with vehicle. Next steps at Cellzome AG include using the <i>in vitro</i> screening platform to identify additional highly selective kinase inhibitors. Cellzome said it discontinued CZC24832 for strategic reasons. SciBX 5(20); doi:10.1038/scibx.2012.533 Published online May 17, 2012	Unpatented; licensing status undisclosed	Bergamini, G. <i>et al. Nat. Chem. Biol.</i> ; published online April 29, 2012; doi:10.1038/nchembio.957 Contact: Gitte Neubauer, Cellzome AG, Heidelberg, Germany e-mail: gitte.neubauer@cellzome.com
Cancer; sepsis	Heat shock protein 90 (Hsp90); lipopolysaccharide (LPS)	Cell culture studies suggest inhibiting the Hsp90-LPS interaction could help treat cancer and LPS-mediated inflammatory responses like sepsis. Peptide-based inhibitors were derived from the N-terminal helix of Hsp90. In murine cell lines, the peptide inhibitors decreased LPS-mediated inflammatory responses compared with a control peptide. In a human breast cancer cell line, a cell-permeable variant of one of the peptides lowered cell viability compared with a control peptide. Next steps include evaluating inhibition of the Hsp90-LPS interaction using <i>in vivo</i> models of cancer and sepsis. At least 14 companies have Hsp90 inhibitors in Phase III testing or earlier to treat various cancers. SciBX 5(20); doi:10.1038/scibx.2012.534 Published online May 17, 2012	Patent application filed covering use in cancer, sepsis and autoimmune diseases; available for licensing	Wu, S. <i>et al. J. Biol. Chem.</i> ; published online April 24, 2012; doi:10.1074/jbc.M112.343848 Contact: Zihai Li, Medical University of South Carolina, Charleston, S.C. e-mail: zihai@muscc.edu

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
Drug platforms			
Antisense oligonucleotides composed of 2'-deoxy-2'-fluoro (2'-F) nucleotides to modulate splicing	Antisense oligonucleotides composed of 2'-F nucleotides could help modulate splicing for therapeutic applications. In human cells, an antisense oligonucleotide composed of 2'-F nucleotides recruited interleukin enhancer binding factor 2 (ILF2) and ILF3, which led to the skipping of exon 7 in <i>survival of motor neuron 2 centromeric (SMN2)</i> . In transgenic <i>SMN2</i> mice, injection of a 2'-F antisense oligonucleotide increased the number of transcriptions with a skipped exon 7 compared with injection of saline. Next steps include exploring potential therapeutic applications of the modified oligonucleotides and looking at other chemical modifications that produce similar effects.	Patent applications filed; available for licensing or partnering for some applications	Rigo, F. <i>et al. Nat. Chem. Biol.</i> ; published online April 15, 2012; doi:10.1038/nchembio.939 Contact: C. Frank Bennett, Isis Pharmaceuticals Inc., Carlsbad, Calif. e-mail: fbennett@isisph.com
	<i>SciBX</i> 5(20); doi:10.1038/scibx.2012.535 Published online May 17, 2012		

CORRIGENDA AND ERRATA

Corrigendum: Analysis: Cover Story

Cain, C. *SciBX* 5(19); doi:10.1038/scibx.2012.483
Published online May 10, 2012

The Analysis item "A mind for precompetitive collaboration" misstated the size of AstraZeneca's neuroscience R&D group. AstraZeneca has not disclosed the size of its neuroscience R&D group.

Company and institution index

A	
Abide Therapeutics Inc.	14
Acucela Inc.	4
Affectis Pharmaceuticals AG	6
AstraZeneca plc	7
B	
Beth Israel Deaconess Medical Center	7
C	
California Institute of Technology	9
Cancer Research Technology Ltd.	7
Cancer Research UK	7
Case Western Reserve University	5
Cellzome AG	15
Cleveland Clinic Lerner College of Medicine of Case Western Reserve University	2
D	
Dana-Farber Cancer Institute	7,11
E	
Elixir Pharmaceuticals Inc.	14
Evotec AG	5,13
G	
GlaxoSmithKline plc	6,9,14
H	
Harvard Medical School	4,7
I	
iVeena Pharmaceuticals Inc.	1,14
M	
Maastricht University	5
Massachusetts General Hospital	7
Mayo Clinic	5
N	
Novartis AG	11
P	
Pfizer Inc.	5
R	
Ruga Corp.	7
S	
Sirtris Pharmaceuticals Inc.	14
T	
Trinity Biomedical Sciences Institute	2
Trinity College Dublin	2,14
U	
University College London Medical School	5
University of Ferrara	5
University of Kentucky	3
University of Texas MD Anderson Cancer Center	7,11

Y	
Yale University	9

Target and compound index

8-Oxo-deoxyguanine	12
8-Oxo-dG	12
A	
ACU-4429	4
AFC-5128	6
AKT	7
AKT1	7
Alu RNA	1
Ampicillin	12
ASC	1
ATP	5
AZ10606120	5
B	
B1R	13
BDKRB1	13
Benzimidazole-pyrimidone	11
Benzoxazole-pyrimidone	11
BEZ235	11
Bradykinin B1 receptor	13
C	
<i>c-Myc</i>	7
CASP1	1
Caspase-1	1
CCNE	11
Cholesterol	1,12
Cyclin E	11
CZC24832	15
D	
DGKE	13
Diacylglycerol kinase-ε	13
DICER1	3
Dicer 1 ribonuclease type III	3
Doxycycline	7
Drusen	1
E	
E3	9
E3 ubiquitin ligase	9
ELA-2	11
EGFR	7
Epidermal growth factor receptor	7
EVT 401	5
F	
FAAH	14
Fatty acid amide hydrolase	14
FLT1	12
FRAP	7,11
G	
GFPT1	7,11
Glutamine-fructose-6-phosphate transaminase 1	7,11
GSK1482160	6
H	
Heat shock protein 90	15
Hsp90	15
Htt	13

Huntingtin	13
Hydroxyflavone	12
I	
IDH1	7
IL-1β	1,5
IL-6	6
IL-18	1,5,14
ILF2	16
ILF3	16
Immunoglobulin light chain	15
Influenza A virus PB1-F2 protein	13
Interleukin enhancer binding factor 2	16
Islet amyloid peptide aggregate	1
Iso citrate dehydrogenase 1	7
K	
Kalata B1	13
Kanamycin	12
K-Ras	7,9,11
L	
Lipopolysaccharide	15
LPS	15
M	
MAGL	14
Mammalian target of rapamycin	7,11
MEK	11
MMAC1	7,11
Monoacylglycerol lipase	14
mTOR	11
MYC	7
MYD88	3,14
Myeloid differentiation primary response gene 88	3,14
N	
NALP3	1,14
NE	11
Neutrophil elastase	11
NLR family pyrin domain containing 3	1,14
NLRP	1
NLRP3	1,14
Nod-like receptor protein	1
Norfloxacin	12
O	
O-Aryl carbamate	14
O-Hexafluoroisopropyl (HFIP) carbamate	14
P	
P2RX5	5
P2RX7	5
P2RY1	5
P2RY2	5
P2RY11	5
P2X	5
P2X5	5
P2X7	5
P2Y	5
P2Y1	5
P2Y2	5
P2Y11	5
PB1-F2	13
Perilipin 5	12
Phosphoinositide 3-kinase	7
Phosphoinositide 3-kinase-γ	13,15
Phosphoinositide 3-kinase-β	11
PI-103	11
Pi3Kα	11
PI3Kβ	11
PI3Kγ	13,15
PI3K	7,11,15
PKB	7
PKBA	7
PKM2	7
PLIN5	12
PROTAC	9
<i>Protein kinase B</i>	7
Proteolysis-targeting chimeric molecule	9
PTEN	7,11
Purinergic receptor P2X ligand-gated ion channel 5	5
Purinergic receptor P2Y G protein-coupled 1	5
PYCARD	1
PYD and CARD domain containing	1
Pyruvate kinase M2 isoenzyme	7
R	
RAFT1	7,11
Reactive oxygen species	3,12
Ribose 5-phosphate isomerase A	7,11
Ribulose-5-phosphate-3-epimerase	7
Rpe	7
ROS	3,12
RPIA	11
S	
SIRT1	14
Sirtuin 1	14
SMN2	16
SRT1720	14
Sulfamoylphenyl pyrazine	13
<i>Survival of motor neuron 2 centromeric</i>	16
T	
TEP1	7,11
Th17	15
T helper type 17	15
THP	15
TLR	3
TLR9	12
Toll-like receptor	3
Toll-like receptor 9	12
U	
UMOD	15
Uric acid	1
Uromodulin	15
V	
VEGF	2,5
VEGFR-1	12
VEGF receptor 1	12