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APRIL 3, 2014 • VOLUME 7 / NUMBER 13

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Critical mass in diabetes

By Lauren Martz, Staff Writer

A **University of Bremen** group has found a new target—serine/threonine kinase 4—that can increase β cell mass and could represent an alternative to invasive islet cell transplants in patients with diabetes.[1] Next, the team will need to rule out long-term safety issues associated with blocking the kinase.

Destruction of insulin-secreting β cells occurs in both type 1 and type 2 diabetes but through different mechanisms. In type 1 disease, an autoimmune reaction destroys the pancreatic β cells. In type 2, exhaustion and cellular toxicity are responsible.

Previous clinical studies have suggested that even a small amount of endogenous insulin production can maintain functional β cells and improve patient outcomes. In patients with type 1 diabetes, intensive insulin therapy helps sustain endogenous insulin secretion and decreases the risk of chronic complications.[2]

Another approach in clinical development for patients with type 1 diabetes is islet cell transplants. The problems with the approach are the invasiveness of the procedure and low donor cell survival.

A team led by Kathrin Maedler and Amin Ardestani opted for a different approach—blocking the apoptosis of β cells. The group started with a central player in multiple apoptotic processes—serine/threonine kinase 4 (STK4).

Maedler is head of the islet biology laboratory group at the University of Bremen. Ardestani is junior group leader of the islet biology laboratory group at the university. The paper also included researchers from the **Lille 2 University of Health and Law**, **Novo Nordisk A/S**, **Fudan University** and the **University of Illinois at Chicago**.

The first question was whether STK4 activation had any correlation with β cell apoptosis.

The group found that STK4 activity was upregulated in rat β cells and human and mouse islets under diabetogenic conditions including high glucose levels. The team also saw STK4 activation in islets from humans with type 2 diabetes and from obese, diabetic mice.

To determine the effects of STK4 activity in diabetes, the team overexpressed the kinase in human islets and rat β cells. Adenoviral overexpression of *STK4* induced β cell apoptosis, whereas overexpression of a mutant version that had no kinase activity had no such effects.

Decreasing *STK4* expression protected human islets from apoptosis and improved glucose-stimulated insulin secretion.

Notably, the improvement in insulin secretion could not be completely explained by the increase in β cells. Overexpression

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of *STK4* resulted in a complete loss of glucose-stimulated insulin secretion despite an incomplete loss of β cells, suggesting another STK4-dependent mechanism was at work.

STK4 overexpression also decreased levels of pancreatic and duodenal homeobox 1 (PDX1; IPF1), a transcription factor involved in β cell development and function, compared with GFP overexpression. Decreases in PDX1 are known to alter insulin production and secretion.[3,4]

Thus, STK4 inhibition may help treat diabetes through two mechanisms: preventing β cell apoptosis and restoring insulin production and secretion from the cells.

Finally, the team found that blocking Stk4 activity helped treat mouse models of both type 1 and type 2 diabetes.

In mice with type 1 disease, β cell–specific *Stk4* knockout improved glucose tolerance and preserved Pdx1 function, and it increased β cell mass by increasing proliferation and decreasing apoptosis compared with no alteration. Systemic *Stk4* knockout had similar antidiabetic effects and a similar safety profile.

In a mouse model of high-fat diet–induced type 2 diabetes, β cell–specific knockout of *Stk4* also increased β cell mass and prevented impairments in insulin secretion.

Data were published in *Nature Medicine*.

"The major advantage of STK4 as a drug target is that it harbors the potential to directly interfere with β cell death and dysfunction in type 1 and type 2 diabetes. This would be a considerable advancement over currently available therapeutics that do not address the progressive loss of β cells in type 1 and type 2 diabetes," said Cord Dohrmann, CSO of **Evotec AG**.

Evotec and the **MedImmune LLC** unit of **AstraZeneca plc** are developing EVT 770, a β cell regeneration factor that is in preclinical testing to treat diabetes.

The biotech has ongoing collaborations with **Harvard University** and other pharmaceutical companies that are primarily targeting β cell regenerative mechanisms.

Ole Madsen, senior principal scientist at Novo Nordisk and an honorary professor in diabetes stem cell research at the **University of Copenhagen**, noted that the β cell preservation effect of inhibiting Stk4 was highly potent in mice.

"Reduced STK4 activity not only can improve cell proliferation but also has been shown to facilitate cancer development, which has to be carefully analyzed and excluded in chronic studies using animal models with global or tissue-specific knockout of STK4 before such treatment can be contemplated."

—George Kunos, National Institutes of Health

Novo Nordisk markets several diabetes drugs including the long-acting insulin analogs Tresiba insulin degludec and Levemir insulin detemir and the long-acting glucagon-like peptide-1 (GLP-1) agonist Victoza liraglutide.

In addition to preventing β cell apoptosis in both type 1 and type 2 diabetes, Madsen said that another advantage of inhibiting STK4 is the potential to prevent development of the disease in patients at high risk of type 1 diabetes and in the metabolic syndrome stages of type 2 diabetes.

STKIer for specificity

The challenge will be developing a kinase- and tissue-specific approach to target STK4.

Thomas Frogne, senior scientist of incretin and islet biology at Novo Nordisk and coauthor on the paper, said that there were no obvious side effects caused by global or β cell–specific *Stk4* knockout, which is a good indicator that inhibiting the kinase may be safe. However, he cautioned that *STK4* "is widely expressed, and its global inhibition could be associated with tissue-selective side effects."

Frogne said that the company has no immediate plans to explore other STK4 inhibitors.

Dohrmann said that a challenge associated with disease-modifying strategies focused on antiapoptotic pathways is separating the positive effects from the potential safety issues associated with targeting genes involved in tumor suppression.

"In such cases, it would be of great benefit to selectively target the pancreas or ideally the β cells," Dohrmann added.

George Kunos, a senior investigator at the **NIH**, wanted to see the long-term effects of STK4 inhibition. "Reduced STK4 activity not only can improve cell proliferation but also has been shown to facilitate cancer development, which has to be carefully analyzed and excluded in chronic studies using animal models with global or tissue-specific knockout of STK4 before such treatment can be contemplated," he said.

The patent and licensing status of the work is undisclosed.

Martz, L. *SciBX* 7(13); doi:10.1038/scibx.2014.360
Published online April 3, 2014

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University of Copenhagen, Copenhagen, Denmark
University of Illinois at Chicago, Chicago, Ill.

Bridging the German gap

By Chris Cain, Senior Writer

Evotec AG's purchase of **Bionamics GmbH** gives it access to a collection of autoimmune assets sourced from German universities. The move is the latest in a series of steps taken by Evotec to build a pipeline of new university-generated assets to complement its fee-for-service operations.

The move returns Bionamics CEO Timm Jessen to Evotec in the new position of EVP of business development in charge of EVT Innovate, the business segment that houses the biotech's translational projects and additional discovery partnerships.

The appointment comes 10 years after Jessen departed as CSO of Evotec and 8 years after he formed Bionamics to develop programs that originated from German academics.

"Bionamics was formed in 2006 to pursue the idea that universities don't have to build a company around every asset they want to advance—it can be much more efficient to mature innovations in collaboration with companies and CROs experienced in their respective field," Jessen said. "The original idea of our company was to mature projects via this method, and we ended up working with the German government for public support of such asset maturation."

"Bionamics was formed in 2006 to pursue the idea that universities don't have to build a company around every asset they want to advance—it can be much more efficient to mature innovations in collaboration with companies and CROs experienced in their respective field. The original idea of our company was to mature projects via this method, and we ended up working with the German government for public support of such asset maturation."

—Timm Jessen, Evotec AG

Bionamics' first order of business was creating a public-private consortium called **NEU²** to develop therapies for neurodegenerative diseases, with a focus on multiple sclerosis (MS).

In 2008, NEU² was 1 of 3 consortia to receive an award from the Pharmaceutical Initiative for Germany program, which included a €100 million ($137 million) funding competition for consortia intended to boost life science projects in the country. The initiative was launched in 2007 by Germany's **Federal Ministry of Education and Research** to encourage pharmaceutical R&D in Germany.

Thus far NEU² has received a commitment of €40 million ($55 million) from the German government. This will fund the program through 2017, and Jessen will continue to coordinate it from Evotec.

Any IP is owned by the organization that generates it. Consortium members collaborating on a project and contributing the most private funding receive first option to license the IP. If the option is waived, other consortium members then have the choice to license the IP.

NEU² has grown to 12 members, including academic institutions and German biopharma companies such as **Merck KGaA**, **Biotest AG** and Evotec.

Jessen said that Bionamics' role as coordinator of NEU² helped build a reputation with German and international institutions for helping to secure funding and collaborators for academic projects. These relationships helped Bionamics negotiate successful project participation as well as exclusive licenses for itself for about 10 projects in which the company saw potential to treat multiple autoimmune diseases in addition to MS.

"After about two to three years of doing this, where people were coming to us with interesting MS products, people realized we had an interesting structure where they could get experience in asset management and funding as well as disease and market know-how, and more and more projects came in," said Jessen.

Evotec CEO Werner Lanthaler told *SciBX* that the portfolio of assets was a deal driver. "The biggest misunderstanding of this announcement was that we are acquiring what three people had done. You have to consider the networks and connections built around Bionamics," he said.

Bionamics had three employees at the time of its acquisition. Terms of the deal were not disclosed.

In the last three years, Evotec has adopted a strategy in line with Bionamics' founding mission—forming alliances with academic institutions to help develop new products. These include deals with **Harvard University**, **Yale University** and the **Dana-Farber Cancer Institute**.[1,2]

Since 2011, Evotec has started 11 programs it considers part of EVT Innovate, which it defines as programs focused on first-in-class research investments that are eligible for returns through upfront payments, milestones and royalties.

Lanthaler and Jessen said that the drug discovery capabilities of Evotec will enable in-house advancement of assets that Bionamics might otherwise have had to contract out to third parties.

Jessen said that the programs exclusively licensed by Bionamics are largely undisclosed. He said that one preclinical program from the **University Medical Center Hamburg-Eppendorf** uses nanoparticles to selectively deliver autoantigen peptides to liver cells to enable antigen-specific T_{reg} induction and tolerance to various autoimmune diseases.

Cain, C. *SciBX* 7(13); doi:10.1038/scibx.2014.361
Published online April 3, 2014

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Harvard University, Cambridge, Mass.
Merck KGaA (Xetra:MRK), Darmstadt, Germany
NEU², Hamburg, Germany
University Medical Center Hamburg-Eppendorf, Hamburg, Germany
Yale University, New Haven, Conn.

Narrowing down graft stenosis

By Benjamin Boettner, Associate Editor

One of the dominant problems after coronary artery bypass graft surgery is hyperplastic growth inside the transplanted blood vessel that can lead to stenosis. Now, a team at the **NIH**'s **National Heart, Lung, and Blood Institute** has found that this hyperplasia is rooted in a change in the phenotype of endothelial cells lining the transplanted veins and has shown that blocking transforming growth factor-β activity in mice can significantly slow the cellular changes.[1]

Next, the team will try to replicate the findings using transplant models in pigs and will investigate the transforming growth factor-β (TGFB; TGFβ) pathway for other points of intervention that could represent therapeutic targets.

Cellular changes are a fundamental part of the vascular remodeling process after coronary artery bypass graft (CABG) surgery. The surgery involves grafting a blood vessel—often a vein—to an atherosclerotic coronary artery, thus creating a new route for blood to flow that bypasses the blockage.

However, about 40% of vein grafts fail within two years of surgery, frequently because of adverse vascular remodeling during which cell masses—termed neointima—build up in the walls of the grafted veins. The neointima are formed largely by migrating and proliferating cells that resemble vascular smooth muscle cells (VSMCs).

There are no treatments for prevention of long-term graft failure, and there has been little progress in designing therapies to treat or prevent neointimal hyperplasia because neither the mechanisms controlling neointima formation nor the origins of the cells involved have been well understood.

Now, a team led by Manfred Boehm at the National Heart, Lung, and Blood Institute has used a fluorescence-based vein transplantation model in mice to track changes in endothelial cells in the grafted vessels and has found that endothelial cells take on properties of mesenchymal cells. The endothelial-to-mesenchymal transition yields cells with VSMC-like features and appears to be controlled by TGFβ signaling, which could provide a handle for developing targeted therapies.

Boehm is a senior investigator at the National Heart, Lung, and Blood Institute.

Neointima-te details

To find out whether neointima derive from the graft itself or are formed by VSMCs from distant sites in the recipient mice, Boehm's team removed jugular veins containing fluorescently labeled endothelia from transgenic donor mice and grafted them into the femoral artery of nonfluorescent recipient mice.

Neointima formed following the surgery and contained a large proportion of cells derived from the grafted fluorescent endothelial cells. The number of endothelial lineage cells in the neointima increased throughout the 35-day monitoring period, whereas levels of other cells in the neointima reached a plateau by day 14 post-surgery.

The team then characterized the fluorescent cells from the neointima using markers specific for endothelia, including platelet/endothelial cell adhesion molecule (Pecam1; Cd31) and VE-cadherin (Cd144; cadherin-5), and markers specific for immature VSMCs, including actin α2 smooth aorta muscle (Acta2; α-sma) and transgelin (Tagln; Sm22).

In the first few days after surgery, the neointima cells expressed mainly endothelial markers. But they contained predominantly immature VSMC markers by day 35.

The researchers found similar results in human tissue samples from early phase failed vein grafts, which expressed the immature VSMC markers α-SMA and SM22 in addition to endothelial markers.

They concluded that the grafted endothelial cells had undergone a progressive shift in phenotype—which represented an endothelial-to-mesenchymal transition—and that 85% of the neointimal cells had a mesenchymal phenotype by the end of the observation period.

Next, the team looked for the molecular pathway driving the phenotypic change.

They focused on TGFβ-mediated signaling because the cytokine had previously been implicated in vascular remodeling in developmental and fibrotic processes and is thought to regulate thickening of the endothelial lining after vascular injury.[2,3]

The team found that TGFβ-regulated smad family member 2 (Madh2; Smad2) and Smad3 (Madh3) activated the snail family zinc finger 2 (Snai2; Slug; Snail2) transcription factor to drive the endothelial-to-mesenchymal transition. In addition, shRNA against *Smad2* or *Smad3* in mouse veins prior to performing the graft decreased the formation of neointima by day 35 post-surgery compared with control shRNA.

Finally, the team tested whether blocking the pathway pharmacologically could affect the vascular remodeling. TGFβ-neutralizing antibodies produced smaller neointimal areas and fewer endothelial-derived cells in graft-transplanted mice than control antibodies. The team concluded that inhibiting TGFβ could decrease neointima formation after transplanting vein grafts.

The findings were published in *Science Translational Medicine*.

Of pigs and men

Angela Bradshaw, a research fellow at the **University of Glasgow Institute of Cardiovascular and Medical Sciences**, told *SciBX*, "The study has undoubtedly made an important contribution to our understanding of how TGFβ promotes vein graft neointima formation."

However, she said that further studies on the mechanism are needed because other TGFβ-induced pathways such as the Smad1 (Madh1), Smad5 (Madh5) and Smad9 (Madh9) pathway, which is triggered in endothelial cells by the activin receptor-like kinase 1 (Acvrl1; Alk1; Hht2) receptor, may have opposite effects to Smad2 and Smad3.

Boehm said that his team plans to continue studying the molecular pathway *in vitro* to tease out possible synergies or antagonisms with the aim of identifying an optimal target.

> **"The study has undoubtedly made an important contribution to our understanding of how TGFβ promotes vein graft neointima formation."**
>
> ***—Angela Bradshaw, University of Glasgow Institute of Cardiovascular and Medical Sciences***

"As TGFβ itself is such a multifunctional protein, systemic treatment would have to be kept to a minimum. It would be interesting to test whether pretreatment of vein grafts alone before surgery is sufficient to exert therapeutic or beneficial effects."
—Peter ten Dijke, Leiden University

Peter ten Dijke suggested that researchers test whether pretreatment of the grafts can have therapeutic effects and thereby circumvent the wide range of effects of TGFβ inhibitors when applied systemically. "As TGFβ itself is such a multifunctional protein, systemic treatment would have to be kept to a minimum. It would be interesting to test whether pretreatment of vein grafts alone before surgery is sufficient to exert therapeutic or beneficial effects," he said.

He added that existing drugs that interfere with TGFβ signaling should be tested, such as Cozaar losartan—a systemic inhibitor of angiotensin-induced activation of TGFβ signaling.

ten Dijke is a professor at **Leiden University** and group leader at the **Leiden University Medical Center**, where he studies TGFβ signaling in diverse disease processes.

Cozaar reduced aortic dilation rate in the recent COMPARE (COzaar in Marfan PAtients Reduces aortic Enlargement) trial on patients with Marfan syndrome—a genetic connective tissue disease that affects the heart valves and aorta and is driven by ectopic TGFβ signaling.

Cozaar is an angiotensin II type 1 receptor (AGTR1) antagonist marketed by **Merck & Co. Inc.** to treat hypertension, heart failure and stroke.

ten Dijke and Anita Thomas both told *SciBX* that further studies in additional species would be needed to translate the findings for clinical applications.

According to Thomas, although the mouse model of vein grafting allows lineage tracing, its main disadvantage is that at one month after surgery, the graft is quite different from the vein that was grafted into position.

"Very few of the original cells remain in the graft, and most of the extracellular matrix proteins will have been altered during the cellular repopulation of the graft and vessel wall remodeling," she said. "The use of a large-animal model of vein grafting, such as the carotid artery end-to-end or end-to-side model in the pig, would effectively eliminate this problem since grafts in pig models are much thicker, containing more cell layers and are more stable over time."

Thomas is a research associate at the **University of Bristol School of Clinical Sciences**, where she investigates therapeutic developments in cardiovascular disease.

ten Dijke added that studying endothelial-to-mesenchymal transition in other human pathologies such as cerebellar cavernous malformations, Kaposi sarcoma herpesvirus–induced endothelial transformation or tissue fibrosis could also benefit the understanding of adverse vascular remodeling in CABGs.

Boehm told *SciBX* that his team is suggesting further *in vivo* studies using larger animals including pigs. In addition, he said that the findings can be bolstered by examining changes caused by arteriovenous shunts in patients with kidney disease and patients with peripheral arteriovenous bypasses, who tend to have even worse outcomes than CABG recipients.

The findings have not been patented.

Boettner, B. *SciBX* 7(13); doi:10.1038/scibx.2014.362
Published online April 3, 2014

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Complementing mAbs

By Kai-Jye Lou, Senior Writer

Genmab A/S and **Utrecht University** researchers have shown that hexameric IgG complexes can activate the complement cascade, providing the molecular rationale behind the biotech's HexaBody platform.[1] Genmab is now selecting key mutations and antibody candidates to generate IgG antibodies that potently induce complement-dependent cytotoxicity in cancer and infectious disease.

In complement-dependent cytotoxicity, antigen-bound antibodies activate the complement system to induce the formation of membrane attack complexes on the surface of the target cell. These complexes disrupt the integrity of the cell membrane, resulting in lysis.

Despite being a well-known phenomenon, the mechanistic details of how antibodies activate the complement system were poorly characterized.

Genmab presented preliminary proof-of-concept efficacy data for its HexaBody technology at the **American Society of Hematology** meeting last December. In preclinical leukemia and lymphoma models, IgG antibodies with mutations in the Fc region conferring an enhanced ability to form hexamer structures induced more potent complement-dependent cytotoxicity than unmodified counterparts.

The IgG hexamers are arranged in a ring structure, with the Fc regions of the antibodies pointed toward the center.

At the time, the company did not disclose the mechanistic underpinnings of how enhancing IgG hexamer formation with its HexaBody platform led to improved complement-dependent cytotoxicity.

Now, a group co-led by Utrecht University and Genmab researchers has published in *Science* the details of how IgGs activate the complement system.

The researchers used a blocking peptide and a series of point mutation studies to show that the ability of IgGs to form hexamers is mediated by noncovalent interactions involving the antibody's Fc region. Disrupting the antibody's ability to form hexamers had multiple effects. These included impaired recruitment and activation of complement component 1 q subcomponent (C1q) and decreased ability to induce complement-dependent cytotoxicity.

The researchers then showed that IgGs could be engineered to have enhanced hexamer-forming capabilities. The group engineered prototypical human mAbs from all four IgG subclasses with an E345R point mutation, which is located in the Fc region of IgG.

The mutation improved the antibody's ability to form hexamers upon binding a target antigen. These IgGs formed hexamers that recruited and potently activated C1q (*see* **Figure 1, "Bird's-eye view of IgG hexamers"**).

In a human lymphoma cell line, the E345R-mutant IgGs were better able to activate the complement system and induce complement-dependent cytotoxicity than unmodified isotype controls.

Researchers from **The Scripps Research Institute** also contributed to the study.

"We showed that Fc-Fc contacts are indeed important for the assembly of these antibodies into hexamer structures after they've bound to their target antigen and that it is this hexamer that acts as the docking structure for C1q," said Genmab SVP and scientific director Paul Parren, the principal investigator on the study. "Now that we know how to enhance this hexamer-forming activity, we could potentially engineer antibodies that induce improved killing of target cells, and hopefully that will translate into improved therapeutic efficacy."

"The demonstration that IgG hexamerization after antigen binding is effective for all four human IgG isotypes is particularly relevant and convincing."

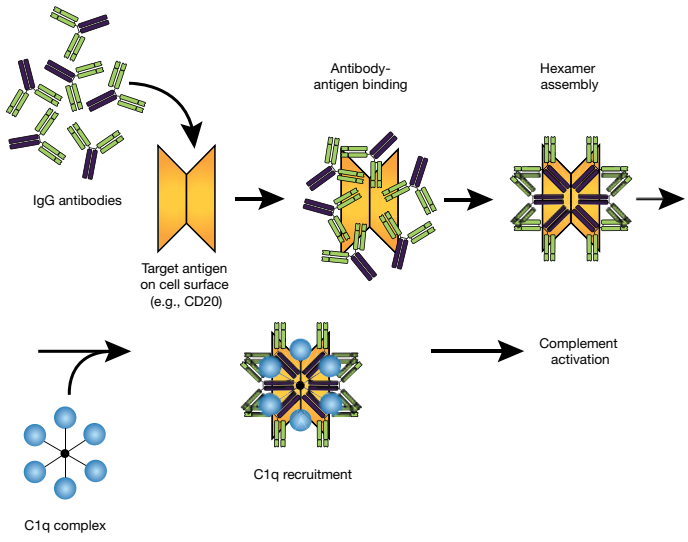
—Alain Beck, Center of Immunology Pierre Fabre

"The demonstration that IgG hexamerization after antigen binding is effective for all four human IgG isotypes is particularly relevant and convincing," said Alain Beck, senior director of antibody and antibody-drug conjugate physico-chemistry at the **Center of Immunology Pierre Fabre**.

He said that the approach thus seems to be generalizable to designing immunotherapeutics with enhanced properties.

"At the atomic level, the findings provide insights on how to dissect and potentially modulate the amount of complement activation," said Barry Springer, VP of technology, strategy and operations for the Biotechnology Center of Excellence at the Janssen Research & Development LLC unit of **Johnson & Johnson**.

Figure 1. Bird's-eye view of IgG hexamers. The complement component 1 q subcomponent (C1q) complex has six antibody-binding headpieces (blue circles) and activates the complement system. Antigen-bound antibodies recruit and activate C1q, but individual IgG antibody molecules bind the complex with weak affinity. As reported by Diebolder *et al.*, monoclonal IgGs can be engineered with point mutations that enhance their ability to assemble into hexamers after binding to a target antigen on the cell surface. This IgG hexamer structure is better able to recruit and activate the C1q complex, leading to increased complement activation and more potent complement-dependent cytotoxicity against the target cell compared with individual IgG molecules.



In 2012, Janssen Biotech Inc. partnered with Genmab to use the biotech's DuoBody technology to create panels of bispecific antibodies against undisclosed disease target combinations selected by Janssen.

Also that year, Genmab granted Janssen exclusive worldwide rights to develop and commercialize daratumumab (HuMax-CD38), a human mAb against CD38, for $55 million up front, up to $1 billion in milestones and tiered, double-digit royalties. The pharma also took a 10.7% percent equity stake in Genmab.

The pharma plans to start Phase III testing of daratumumab to treat relapsed or refractory multiple myeloma (MM) this month.

The pharma does not have a partnership involving HexaBody technology.

Bolstering an insufficient response

A key goal of Genmab's HexaBody technology is to provide a general approach for enhancing the potency of new or existing mAbs without significantly disrupting their native structure and specificity.

Parren and Springer both noted that engineering mAbs with an improved ability to induce complement-dependent cytotoxicity could be useful in situations in which the cell-killing response caused by an existing antibody is insufficient.

Parren said that HexaBody technology could be used to augment the effect of existing therapeutic mAbs that kill target cells via other mechanisms, such as the blockade of a key receptor or antibody-dependent cell-mediated cytotoxicity.

"Some pathogen and tumor cell antigens have low presentation or density on the cell surface, which could limit the ability of native antibodies" to kill the cell, added Springer. "The current study suggests they could now potentially bypass such issues by specifically tailoring antibodies that have a propensity to aggregate and promote complex formation."

According to Parren, the mechanism described in the paper suggests that mAbs engineered with the HexaBody platform would induce complement-dependent cytotoxicity in a highly specific and localized manner.

"The hexamer formation process only happens once the antibody binds to the target antigen. The IgGs otherwise remain in a monomeric state while in circulation that cannot interact with C1q," he said.

Parren declined to disclose the specific complement-enhancing mutations and mAbs that Genmab plans to use for its HexaBody platform but did note that the E345R mutation described in the study is just a prototype complement-enhancing mutation and not one of the mutations the company expects to use.

"The *Science* paper helps to set the stage for others to understand what the potential of our HexaBody technology could be. We are definitely thinking about how to use this technology to generate new candidates for our pipeline," he added.

Springer said that it will be important to determine the extent to which enhancing complement activity could promote tumor cell killing and how incorporating improved complement activation affects therapeutic mAbs that already kill their target cells via other antibody-mediated mechanisms such as antibody-dependent cell-mediated cytotoxicity.

He added that it also will be important to show that it is possible to do scaled-up manufacturing of the complement-activating mAbs.

Genmab has filed multiple patents covering various aspects of its HexaBody technology. The technology is available for licensing and partnering.

Lou, K.-J. *SciBX* 7(13); doi:10.1038/scibx.2014.363
Published online April 3, 2014

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1. Diebolder, C.A. *et al. Science*; published online March 13, 2014; doi:10.1126/science.1248943

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COMPANIES AND INSTITUTIONS MENTIONED

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Center of Immunology Pierre Fabre, St. Julien-en-Genevois, France
Genmab A/S (CSE:GEN; OTCBB:GMXAY), Copenhagen, Denmark
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
The Scripps Research Institute, La Jolla, Calif.
Utrecht University, Utrecht, the Netherlands

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				
Acute lymphocytic leukemia (ALL)	Deoxycytidine kinase (DCK)	*In vitro* and mouse studies suggest simultaneously inhibiting two deoxycytidine triphosphate (dCTP) synthesis pathways could help treat ALL. In human T cell ALL lines, thymidine (dT) decreased dCTP synthesis through the *de novo* pathway but promoted dCTP synthesis through the nucleotide salvage pathway. In human T cell ALL lines, the combination of dT plus a DCK inhibitor that blocks the nucleotide salvage pathway resulted in increased apoptosis and replication stress compared with either compound alone. In mice, the combination decreased tumor burden in human T cell ALL xenografts and leukemic burden in systemic B cell ALL xenografts compared with either treatment alone. Next steps include assessing safety and toxicology. ***SciBX* 7(13); doi:10.1038/scibx.2014.364** **Published online April 3, 2014**	Patent application filed for the DCK inhibitors; unlicensed	Nathanson, D.A. *et al. J. Exp. Med.*; published online Feb. 24, 2014; doi:10.1084/jem.20131738 **Contact:** Caius G. Radu, University of California, Los Angeles, Calif. e-mail: cradu@mednet.ucla.edu
Acute promyelocytic leukemia (APL)	Retinoic acid receptor-α (RARA); promyelocytic leukemia (PML); histone deacetylase (HDAC)	Cell culture studies suggest hybrid retinoic acid–HDAC inhibitors could be useful for treating APL. More than 95% of APLs are driven by PML-RARA fusions, and although retinoic acid or HDAC inhibitors elicit apoptosis in APL cells, nonspecific gene expression changes are known to limit their utility. In cell culture assays, low micromolar concentrations of a hybrid compound consisting of all-trans retinoic acid and an HDAC class I inhibitor maintained partial retinoid activity and induced acetylation at only a subset of PML-RARA target genes. In cell culture, the hybrid compound induced proapoptotic genes and increased caspase-8 (CASP8; FLICE)- and reactive oxygen species–dependent cell death compared with an HDAC inhibitor alone. Next steps include further investigating the hybrid compound's mechanism of action. ***SciBX* 7(13); doi:10.1038/scibx.2014.365** **Published online April 3, 2014**	Unpatented; licensing status not applicable	De Bellis, F. *et al. Cancer Res.*; published online Feb. 24, 2014; doi:10.1158/0008-5472.CAN-13-2568 **Contact:** Lucia Altucci, Second University of Naples, Naples, Italy e-mail: lucia.altucci@unina2.it

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Brain cancer	*Mitogen-activated protein kinase kinase 4* (*MAP2K4*; *MKK4*)	Cell culture and mouse studies suggest small molecules that induce vacuolization could be used to treat glioblastoma multiforme (GBM). Phenotypic screening of a compound library followed by cell-based and zebrafish assays and *in silico* analyses identified the quinine derivative vacquinol-1, which induced vacuolization and cell rupture in human glioma cells. An shRNA screen identified *MKK4* as a gene required for vacquinol-1-induced vacuolization. In mice with established human GBM, oral dosing of vacquinol-1 attenuated tumor growth and enhanced survival. Next steps include determining a dosing regimen, performing toxicity studies and setting up clinical trials with vacquinol-1 or optimized structural analogs. ***SciBX* 7(13); doi:10.1038/scibx.2014.366** **Published online April 3, 2014**	Patent application filed; available for licensing from Karolinska Institute Innovations AB **Contact:** Edwin Johnson, Karolinska Institute Innovations AB, Solna, Sweden e-mail: edwin.johnson@kiinnovations.se	Kitambi, S.S. *et al. Cell*; published online March 18, 2014; doi:10.1016/j.cell.2014.02.021 **Contact:** Patrik Ernfors, Karolinska Institute, Stockholm, Sweden e-mail: patrik.ernfors@ki.se
Breast cancer	G protein–coupled receptor 161 (GPR161)	Patient sample and cell culture studies suggest targeting GPR161 could be useful for treating triple-negative breast cancer (TNBC). In patient breast tumor samples, RNA sequence data showed that GPR161 was overexpressed in TNBC samples, and computational analysis showed that high GPR161 expression correlated with decreased time to relapse. In cultured breast cancer cells, overexpression of GPR161 activated the mammalian target of rapamycin (mTOR; FRAP; RAFT1) signaling pathway and increased proliferation and invasiveness compared with low or no GPR161 expression. Next steps include developing a mouse model to test GPR161-targeted therapeutics and demonstrate a direct role for the receptor in tumorigenesis. ***SciBX* 7(13); doi:10.1038/scibx.2014.367** **Published online April 3, 2014**	Unpatented; licensing status not applicable	Feigin, M.E. *et al. Proc. Natl. Acad. Sci. USA*; published online March 5, 2014; doi:10.1073/pnas.1320239111 **Contact:** Senthil K. Muthuswamy, University of Toronto, Toronto, Ontario, Canada e-mail: s.muthuswamy@utoronto.ca **Contact:** Michael E. Feigin, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. e-mail: feiginm@cshl.edu
Cancer	Cannabinoid CB_2 receptor (CNR2)	*In vitro* and mouse studies suggest a CNR2-specific ligand–based photosensitizer could be useful for photodynamic cancer therapy. In mouse astrocytoma cells engineered to express CNR2, light-irradiated IR700DX-mbc94, a near-infrared phthalocyanine dye coupled to a CNR2-specific ligand, caused markedly more cell death than the nonirradiated dye. In mice with subcutaneous tumors generated from *Cnr2*$^+$ mouse astrocytoma cells, IR700DX-mbc94 plus irradiation decreased tumor growth compared with no treatment. Next steps include reproducing the results with human cancer cells that overexpress CNR2 and improving affinity of the photosensitizer. ***SciBX* 7(13); doi:10.1038/scibx.2014.368** **Published online April 3, 2014**	Invention disclosure filed with the University of Pittsburgh; licensed to an undisclosed entity	Zhang, S. *et al. Chem. Bio.*; published online Feb. 27, 2014; doi:10.1016/j.chembiol.2014.01.009 **Contact:** Mingfeng Bai, University of Pittsburgh, Pittsburgh, Pa. e-mail: baim@upmc.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	Heat shock protein 90 (Hsp90)	*In vitro* and mouse studies suggest a new family of triazole-based Hsp90 inhibitors could help treat cancer. Chemical and cell culture studies identified 1,4,5-trisubstituted triazole carboxylic acid derivatives that targeted the Hsp90 ATP binding site with nanomolar affinities and decreased levels of Hsp90 client proteins and increased levels of Hsp70 and apoptosis in tumor cell lines compared with vehicle. In mouse xenograft models of epidermoid and gastric carcinoma, the best compounds inhibited tumor growth by about 40% at 6–10-fold lower doses than the Hsp90 inhibitor AUY922. Next steps include evaluating safety of the lead compound. Vernalis plc and Novartis AG have AUY922 in Phase II trials to treat hematological and solid tumors. At least 10 other companies have Hsp90 inhibitors in preclinical and clinical development to treat cancer. ***SciBX* 7(13); doi:10.1038/scibx.2014.369** **Published online April 3, 2014**	Patented by Sigma-Tau Group; available for licensing	Taddei, M. *et al. J. Med. Chem.*; published online March 3, 2014; doi:10.1021/jm401536b **Contact:** Giuseppe Giannini, Sigma-Tau Group, Pomezia, Italy e-mail: giuseppe.giannini@sigma-tau.it **Contact:** Maurizio Taddei, University of Siena, Siena, Italy e-mail: maurizio.taddei@unisi.it
Cancer	IL-23; CD40	Mouse studies suggest combination therapy with IL-23 and CD40 mAbs could be more effective at treating cancer than monotherapy. In a mouse model of chemically induced fibrosarcoma, mAbs targeting the IL-23 p19 subunit (IL-23p19) and CD40 delayed tumor onset and decreased tumor incidence compared with either agent alone or control antibody. In mouse models of melanoma- or prostate cancer–derived lung metastasis, the mAb combination decreased tumor incidence. In a mouse model of spontaneous mammary carcinoma metastasis following primary tumor resection, the mAb combination increased survival. Next steps could include optimizing antibody ratios in preclinical models of cancer. Antibodies used in the study were obtained from Amgen Inc., which participated in the study. Amgen and partner AstraZeneca plc have the IL-23-targeted mAb AMG 139 in Phase II testing to treat inflammatory diseases. Bristol-Myers Squibb Co. and Johnson & Johnson market ustekinumab, a human mAb inhibiting IL-12 and IL-23, to treat psoriasis. At least 10 other companies have IL-23-targeting antibodies in Phase III or earlier testing to treat autoimmune diseases or cancer. At least eight companies have CD40-targeting antibodies in Phase II or earlier testing to treat autoimmune diseases and cancer. ***SciBX* 7(13); doi:10.1038/scibx.2014.370** **Published online April 3, 2014**	Patent and licensing status unavailable	Von Scheidt, B. *et al. Cancer Res.*; published online Feb. 20, 2014; doi:10.1158/0008-5472.CAN-13-1646 **Contact:** Michele W.L. Teng, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia e-mail: michele.teng@qimr.edu.au

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Melanoma; prostate cancer; colon cancer	CTLA-4 (CD152)	Mouse studies suggest combining oncolytic Newcastle disease virus (NDV) and CTLA-4 inhibitors could help treat melanoma and other solid tumor types. In mice bearing melanoma tumors in each flank, NDV injection into tumors in one flank increased tumor antigen–specific $CD8^+$ T cell infiltration and decreased tumor growth in both flanks compared with vehicle injection. In mice bearing bilateral melanoma, prostate or colon tumors, NDV injection into tumors in one flank plus a systemic antibody against mouse Ctla-4 decreased bilateral tumor growth and increased survival compared with injection of either agent alone. Ongoing work includes testing an NDV vector expressing undisclosed genes in the melanoma models. Bristol-Myers Squibb Co. markets Yervoy ipilimumab (BMS-734016), a human mAb against CTLA-4, to treat melanoma. The pharma also has the mAb in Phase III testing to treat prostate cancer and Phase II testing to treat non–small cell lung cancer (NSCLC), pancreatic cancer and solid tumors. Pfizer Inc. and AstraZeneca plc have tremelimumab (CP-675; CP-675206), a human mAb against CTLA-4, in Phase II testing to treat liver cancer and solid tumors and Phase I trials to treat melanoma and prostate cancer. Amgen Inc. has the combination of Talimogene laherparepvec (OncoVEX GM-CSF), a modified herpes simplex virus type 1 (HSV-1) encoding granulocyte macrophage colony-stimulating factor (GM-CSF; CSF2), and Yervoy in Phase Ib/II testing to treat melanoma. Amgen also has Talimogene monotherapy in Phase III testing to treat melanoma. ***SciBX* 7(13); doi:10.1038/scibx.2014.371** **Published online April 3, 2014**	Patented by the Icahn School of Medicine at Mount Sinai and the Memorial Sloan-Kettering Cancer Center; available for licensing or partnering	Zamarin, D. *et al. Sci. Transl. Med.*; published online March 5, 2014; doi:10.1126/scitranslmed.3008095 **Contact:** Dmitriy Zamarin, Memorial Sloan-Kettering Cancer Center, New York, N.Y. e-mail: zamarind@mskcc.org **Contact:** James P. Allison, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: jallison@mdanderson.org
Prostate cancer	Ubiquitin specific peptidase 9 X-linked (USP9X; FAF); v-ets erythroblastosis virus E26 oncogene homolog (ERG)	*In vitro*, human tissue and mouse studies suggest inhibiting USP9X could help treat prostate cancer. In *in vitro* assays, the deubiquitinase USP9X bound to and stabilized ERG. In human prostate samples, USP9X expression was higher in ERG^+ prostate tumors than ERG^- tumors and benign tissue. In multiple mouse models of human prostate cancer, pharmacological inhibition of USP9X decreased ERG levels and growth of ERG^+ tumors compared with vehicle treatment. Next steps include developing USP9X inhibitors with improved potency. ***SciBX* 7(13); doi:10.1038/scibx.2014.372** **Published online April 3, 2014**	Unpatented; licensing status not applicable	Wang, S. *et al. Proc. Natl. Acad. Sci. USA*; published online March 3, 2014; doi:10.1073/pnas.1322198111 **Contact:** Ralf Kittler, The University of Texas Southwestern Medical Center, Dallas, Texas e-mail: ralf.kittler@utsouthwestern.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cardiovascular disease				
Coronary artery bypass graft (CABG) surgery	Transforming growth factor-β1 (TGFB1)	Mouse studies suggest local inhibition of TGFB1 signaling could help prevent stenosis of grafted veins after CABG surgery. In mice receiving jugular vein grafts, pretreatment of recipient mice and prepared donor veins with a neutralizing pan-TGFβ (TGFB) antibody decreased the endothelial-to-mesenchymal transition of occluding cells and neointima formation up to 35 days after surgery compared with IgG control pretreatment. In human vein grafts analyzed postmortem, markers indicating ongoing endothelial-to-mesenchymal transition in neointimas were elevated, suggesting conservation of a TGFβ-dependent pathological mechanism. Next steps include further investigating contributions of TGFβ signaling elements and testing TGFβ inhibition in larger animal models. At least 10 companies have TGFB1 inhibitors in Phase II or earlier development to treat inflammatory and fibrotic indications as well as different cancers (*see* **Narrowing down graft stenosis , page 5**). ***SciBX* 7(13); doi:10.1038/scibx.2014.373** **Published online April 3, 2014**	Unpatented; licensing status not applicable	Cooley, B.C. *et al. Sci. Transl. Med.*; published online March 12, 2014; doi:10.1126/scitranslmed.3006927 **Contact:** Manfred Boehm, National Heart, Lung, and Blood Institute, Bethesda, Md. e-mail: boehmm@nhlbi.nih.gov
Endocrine/metabolic disease				
Diabetes	Serine/threonine kinase 4 (STK4)	*In vitro* and mouse studies suggest inhibiting STK4 could help treat diabetes. In human and mouse islets and in rat β cells under diabetic conditions, compared with cells under normal conditions, STK4 activation was increased. In human islets, adenovirus-mediated overexpression of STK4 induced β cell apoptosis, and STK4-targeting siRNA increased β cell survival and function compared with scrambled siRNA. In mouse models of chemical- and high-fat diet–induced diabetes, mice with β cell–specific knockdown of *Stk4* had greater β cell function and β cell mass than wild-type mice and showed improved glucose tolerance. Next steps could include developing an STK4 inhibitor (*see* **Critical mass in diabetes, page 1**). ***SciBX* 7(13); doi:10.1038/scibx.2014.374** **Published online April 3, 2014**	Patent and licensing status undisclosed	Ardestani, A. *et al. Nat. Med.*; published online March 16, 2014; doi:10.1038/nm.3482 **Contact:** Kathrin Maedler, University of Bremen, Bremen, Germany e-mail: kmaedler@uni-bremen.de **Contact:** Amin Ardestani, same affiliation as above e-mail: ardestani.amin@gmail.com

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Glycosphingolipid storage disorders	Not applicable	*In vitro* studies suggest 2-hydroxypropyl-β-cyclodextrin (HPβCD) could help treat lysosomal storage disorders. HPβCD has been used to improve drug stability and bioavailability, but there is evidence that the compound has activity against cholesterol storage disorders. In HeLa cells, HPβCD activated transcription factor EB (TFEB), which is involved in lysosomal regulation, and induced autophagy. In fibroblasts from a patient with the late infantile neonatal ceroid lipofuscinosis lysosomal storage disease, HPβCD improved clearance of ceroid lipopigment, which accumulated in patient cells, through TFEB activation and autophagy. Next steps could include testing the effects of HPβCD in additional storage disorders. The NIH has HPβCD in Phase I trials to treat Niemann-Pick disease type C1 (NPC1). ***SciBX* 7(13); doi:10.1038/scibx.2014.375** **Published online April 3, 2014**	Patent and licensing status unavailable	Song, W. *et al. J. Biol. Chem.*; published online Feb. 20, 2014; doi:10.1074/jbc.M113.506246 **Contact:** Laura Segatori, Rice University, Houston, Texas e-mail: segatori@rice.edu
Infectious disease				
HIV/AIDS	HIV integrase	Macaque studies suggest postcoital dosing of gel-formulated HIV integrase inhibitors could prevent HIV infection. In macaques vaginally challenged twice weekly for 2.5 months with a pathogenic simian-human immunodeficiency virus (SHIV), 5 of 6 receiving vaginal 1% raltegravir gel 3 hours after SHIV exposure remained uninfected, whereas 4 of 4 receiving vaginal placebo gel became infected. Next steps include evaluation of additional integrase inhibitors and modalities for HIV prevention. Merck & Co. Inc. markets the oral HIV integrase inhibitor Isentress raltegravir to treat HIV. Japan Tobacco Inc. and Gilead Sciences Inc. market the integrase inhibitor Vitekta elvitegravir to treat HIV. ViiV Healthcare Ltd. has the integrase inhibitor GSK1265744 in Phase II trials and markets the integrase inhibitor Tivicay dolutegravir to treat HIV. ***SciBX* 7(13); doi:10.1038/scibx.2014.376** **Published online April 3, 2014**	Patent application filed; available for licensing from the NIH **Contact:** Whitney Blair, National Institutes of Health, Bethesda, Md. e-mail: blairw2@mail.nih.gov	Dobard, C. *et al. Sci. Transl. Med.*; published online March 12, 2014; doi:10.1126/scitranslmed.3007701 **Contact:** Walid Heneine, Centers for Disease Control and Prevention, Atlanta, Ga. e-mail: wmh2@cdc.gov

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Musculoskeletal disease				
Musculoskeletal disease	Calcium release–activated calcium channel (CRAC); stromal interaction molecule 1 (STIM1); transmembrane protein 142A (ORAI1; TMEM142A; CRACM1)	*In vitro* and genetic studies suggest decreasing calcium influx through CRAC could help treat Stormorken syndrome and related diseases. Stormorken syndrome is a tubular myopathy with symptoms that include abnormal bleeding. Sequencing studies identified the p.R304M mutation in *STIM1* in two patients with Stormorken syndrome and the p.P2452 mutation in *ORAI1* in two patients with a Stormorken-like syndrome that lacked bleeding symptoms. In human embryonic kidney cells, expression of the *STIM1* mutation caused constitutive activation of CRAC and expression of the *ORAI1* mutation also increased calcium influx by suppressing channel inactivation. Next steps could include developing specific CRAC inhibitors. CalciMedica Inc.'s CRAC inhibitor CM2489 is in Phase I testing to treat psoriasis. Rhizen Pharmaceuticals S.A. and Synta Pharmaceuticals Corp. have CRAC inhibitors in preclinical development for autoimmune and inflammatory indications. ***SciBX* 7(13); doi:10.1038/scibx.2014.377** **Published online April 3, 2014**	Patent status not applicable; unavailable for licensing	Nesin, V. *et al. Proc. Natl. Acad. Sci. USA*; published online March 3, 2014; doi:10.1073/pnas.1312520111 **Contact:** Leonidas Tsiokas, University of Oklahoma Health Sciences Center, Oklahoma City, Okla. e-mail: leonidas-tsiokas@ouhsc.edu **Contact:** Klaas J. Wierenga, same affiliation as above e-mail: klaas-wierenga@ouhsc.edu
Neurology				
Fragile X syndrome	Fragile X mental retardation 1 (FMR1)	*In vitro* studies suggest inhibiting the binding of CGG repeat–containing *FMR1* transcripts to the *FMR1* promoter could help treat fragile X syndrome. Fragile X syndrome is caused by a CGG trinucleotide expansion of more than 200 repeats that are adjacent to the *FMR1* promoter and that silence *FMR1*, but the mechanism of silencing is unknown. In human embryonic stem cells from patients with fragile X syndrome, knockdown of *FMR1* mRNA or a small molecule that binds the CGG-containing mRNA blocked silencing of the gene. In the patient cells, the CGG repeat region of *FMR1* mRNA directly bound the promoter region of *FMR1* to induce silencing. Next steps include assessing the safety of the small molecule. ***SciBX* 7(13); doi:10.1038/scibx.2014.378** **Published online April 3, 2014**	Patent applications filed covering the small molecule; available for licensing	Colak, D. *et al. Science*; published online Feb. 28, 2014; doi:10.1126/science.1245831 **Contact:** Samie R. Jaffrey, Weill Cornell Medical College, New York, N.Y. e-mail: srj2003@med.cornell.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Neurology	CC chemokine receptor 2 (CCR2; CD192); monocyte chemoattractant protein-1 (MCP-1; CCL2)	Studies in patient samples and mice suggest depleting *CCR2*$^+$ inflammatory monocytes could help treat intracerebral hemorrhage. Mice subjected to intracerebral hemorrhage showed higher levels of *Ccr2*$^+$ inflammatory monocytes than sham-operated controls. In patient serum samples, high levels of the CCR2 ligand CCL2 were associated with increased disability and risk of death following intracerebral hemorrhage. In mice, treatment with an anti-CCR2 antibody decreased motor deficits following intracerebral hemorrhage compared with an isotype control antibody. Next steps include evaluating how depletion of inflammatory monocytes affects long-term outcomes after intracerebral hemorrhage and susceptibility to infections. At least six companies have CCR2-inhibiting compounds in Phase II or earlier testing for various conditions outside of neurology. ***SciBX* 7(13); doi:10.1038/scibx.2014.379** **Published online April 3, 2014**	Unpatented; licensing status not applicable	Hammond, M.D. *et al. J. Neurosci.*; published online March 12, 2014; doi:10.1523/JNEUROSCI.4070-13.2014 **Contact:** Lauren Sansing, University of Connecticut Health Center, Farmington, Conn. e-mail: sansing@uchc.edu
Stroke	Phosphoinositide 3-kinase-δ (PI3Kδ)	*In vitro* and mouse studies suggest inhibiting PI3Kδ could help treat stroke. Tumor necrosis factor-α (TNF-α)-mediated inflammation associated with reperfusion after stroke can cause tissue damage. In a microglia-based cell culture model of stroke, the PI3Kδ-selective inhibitor idelalisib (formerly CAL-101) decreased TNF-α secretion compared with vehicle. In a mouse model of stroke, treatment with CAL-101 15 minutes before or up to 3 hours after reperfusion decreased brain damage compared with vehicle. Next steps include preclinical and clinical studies of selective PI3Kδ inhibitors in stroke. Gilead Sciences Inc.'s idelalisib is under regulatory review to treat non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL). Takeda Pharmaceutical Co. Ltd. has the PI3Kδ inhibitor IPI-145 in Phase III testing to treat CLL. At least six other companies have PI3Kδ inhibitors in Phase II testing or earlier to treat various diseases. ***SciBX* 7(13); doi:10.1038/scibx.2014.380** **Published online April 3, 2014**	Unpatented; licensing status not applicable	Low, P.C. *et al. Nat. Commun.*; published online March 14, 2014; doi:10.1038/ncomms4450 **Contact:** Frédéric A. Meunier, The University of Queensland, Brisbane, Queensland, Australia e-mail: f.meunier@uq.edu.au **Contact:** Pei Ching Low, National University of Singapore, Singapore e-mail: phstva@nus.edu.sg

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
Disease models			
Bioluminescent mouse model of HPV⁺ oral tumors to track the effect of therapeutics	A bioluminescent mouse model of HPV⁺ oral tumors could help evaluate potential therapeutic candidates. In mice with inducible expression of the HPV oncogenes *E6 transforming protein* (*human papillomavirus-16*; *HpV16gp1*) and *E7 transforming protein* (*human papillomavirus-16*; *HpV16gp2*) and luciferase, oncogenic K-Ras (Kras) expression in the epithelia induced the development of oral tumors with enhanced bioluminescence that correlated with tumor growth. In the mouse model, rapamycin or image-guided radiotherapy induced tumor regression and decreased bioluminescence at least threefold compared with vehicle or no irradiation. Next steps could include using the model to test preclinical therapeutic candidates. Rapamycin is a generic small molecule inhibitor of mammalian target of rapamycin (mTOR; FRAP; RAFT1). ***SciBX* 7(13); doi:10.1038/scibx.2014.381** **Published online April 3, 2014**	Patent and licensing status unavailable	Zhong, R. *et al. Cancer Res.*; published online Feb. 13, 2014; doi:10.1158/0008-5472.CAN-13-2993 **Contact:** Michael Spiotto, The University of Chicago, Chicago, Ill. e-mail: mspiotto@radonc.uchicago.edu
Mouse model of dominant Ullrich congenital muscular dystrophy (UCMD)	A mouse model of dominant UCMD with deletion of *collagen type VI α3* (*Col6a3*) exon 16 could help identify treatments for the disease. In mice, heterozygous deletion of exon 16 of *Col6a3* resulted in production of both normal and mutant *Col6a3* mRNA, which prevented assembly of collagen microfibrils and mimicked the human condition. The mutant mice had myopathy, ultrastructural changes in muscle mitochondria and sarcoplasmic reticulum, abnormal collagen fibers in tendons and impaired muscle function and contractions. Next steps could include using the model to identify therapeutics. ***SciBX* 7(13); doi:10.1038/scibx.2014.382** **Published online April 3, 2014**	Unpatented; available for licensing	Pan, T.-C. *et al. J. Biol. Chem.*; published online Feb. 22, 2014; doi:10.1074/jbc.M114.549311 **Contact:** Mon-Li Chu, Thomas Jefferson University, Philadelphia, Pa. e-mail: mon-li.chu@jefferson.edu
Drug delivery			
Engineered, zinc finger domain–containing recombinases for site-specific delivery of therapeutic genes	An engineered recombinase could enable nontoxic, site-specific delivery of therapeutic genes to the human genome. Recombinases containing zinc finger, DNA-binding domains form heterodimers capable of site-specific gene delivery but also form homodimers that can modify off-target genes. In a human cell line, a recombinase containing an engineered variant of the zinc finger domain exhibited over 500-fold less homodimerization than a recombinase containing the unmodified domain. In the cell line, the engineered recombinase was used to deliver human *factor IX* or *α-galactosidase A* into the genome with no signs of off-target delivery. Future studies could include developing viral vectors for cell- or tissue-targeted *in vivo* delivery of the engineered recombinase. ***SciBX* 7(13); doi:10.1038/scibx.2014.383** **Published online April 3, 2014**	Patent and licensing status unavailable	Gaj, T. *et al. J. Am. Chem. Soc.*; published online March 10, 2014; doi:10.1021/ja4130059 **Contact:** Carlos F. Barbas III, The Scripps Research Institute, La Jolla, Calif. e-mail: carlos@scripps.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Drug platforms			
Crystal structure of metabotropic glutamate receptor subtype 1 (mGluR1; GRM1)	The crystal structure of mGluR1 could help researchers design mGluR1-targeted therapeutics to treat cancer and various neurological indications. *In vitro*, an X-ray crystal structure of the dimeric, seven-transmembrane domain of mGluR1 bound to a negative allosteric modulator revealed that the overall fold of the transmembrane domain and the modulator binding site resembled the inactive conformation of other classes of G protein–coupled receptors. In this structure, six cholesterols mediated contact between dimerizing receptor subunits, suggesting cholesterol could mediate communication between the extracellular domain that initiates dimerization and the transmembrane domain. Next steps include understanding allosteric mechanisms governing receptor activity and using the structure to optimize allosteric modulators. ***SciBX* 7(13); doi:10.1038/scibx.2014.384** **Published online April 3, 2014**	Unpatented; licensing status not applicable	Wu, H. *et al. Science*; published online March 6, 2014; doi:10.1126/science.1249489 **Contact:** Raymond C. Stevens, The Scripps Research Institute, La Jolla, Calif. e-mail: stevens@scripps.edu
Hexamer-forming mAbs that activate the complement system	mAbs engineered with mutations that promote hexamer formation could help treat infections and cancer by activating the complement system. The complement system is a part of the innate immune system that helps to eliminate pathogens and tumor cells. In cell culture, multiple IgGs engineered with an E345R mutation had greater complement-dependent cytotoxicity than unmodified variants. *In vitro*, IgGs with the E345R mutation were better than control IgGs at forming hexameric structures that activated complement component 1 q subcomponent (C1q) after binding to their target antigen. Next steps include selecting complement activation–enhancing mutations and specific mAb variants to use in Genmab A/S' HexaBody platform for generating therapeutic antibodies. Genmab uses its HexaBody platform to design mAbs that have an improved ability to eliminate pathogens and tumor cells while retaining their regular structure and specificity (*see* **Complementing mAbs, page 7**). ***SciBX* 7(13); doi:10.1038/scibx.2014.385** **Published online April 3, 2014**	Patent application filed; available for licensing and partnering	Diebolder, C.A. *et al. Science*; published online March 14, 2014; doi:10.1126/science.1248943 **Contact:** Paul W.H.I. Parren, Genmab A/S, Utrecht, the Netherlands e-mail: p.parren@genmab.com **Contact:** Piet Gros, Utrecht University, Utrecht, the Netherlands e-mail: p.gros@uu.nl
Mouse cardiomyocytes generated from fibroblasts with one pluripotency factor and four small molecules	Cell culture studies suggest cardiomyocytes for use in pharmacological screening and regenerative therapies could be generated with minimal genetic manipulation. In mouse embryonic and tail tip fibroblasts, expression of the pluripotency transcription factor Oct4 plus a combination of four small molecules led to beating cardiomyocytes via a cardiac precursor state without transition through a pluripotent state. The cardiomyocytes expressed cardiac- and ventricular-specific markers and exhibited a cross-striated pattern and electrophysiological ventricular characteristics. Next steps include isolating and expanding a cardiac cell population and translating the approach to human cardiomyocytes. ***SciBX* 7(13); doi:10.1038/scibx.2014.386** **Published online April 3, 2014**	Patent application filed; available for licensing	Wang, H. *et al. Cell Rep.*; published online Feb. 20, 2014; doi:10.1016/j.celrep.2014.01.038 **Contact:** Sheng Ding, Gladstone Institute of Cardiovascular Disease, San Francisco, Calif. e-mail: sheng.ding@gladstone.ucsf.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Imaging			
Multiplexed ion beam imaging (MIBI) for detection of up to 100 target proteins in clinical samples	Imaging studies suggest MIBI could simultaneously detect up to 100 antigen targets for clinical diagnostics. MIBI involves incubation of biological samples with antibodies coupled to lanthanides, which release secondary ions when exposed to an ion beam. The ions are detected by a magnetic sector mass spectrometer that analyzes each isotope and measures levels of each target. In peripheral blood mononuclear cells, staining with several coupled antibodies generated signals with intensities similar to those for mass spectrometry. In formalin-fixed, paraffin-embedded human breast tumor specimens, MIBI simultaneously and quantitatively detected 10 antigen labels. Next steps include prototype construction and validation. *SciBX* 7(13); doi:10.1038/scibx.2014.387 Published online April 3, 2014	Patent application filed; available for licensing	Angelo, M. *et al. Nat. Med.*; published online March 2, 2014; doi:10.1038/nm.3488 **Contact:** Garry P. Nolan, Stanford University, Stanford, Calif. e-mail: gnolan@stanford.edu
Markers			
Fluorescent antibodies to membrane-bound tumor necrosis factor (mTNF) as a predictive biomarker for therapeutic responders	Fluorescent antibodies against mTNF could provide an *in vivo* molecular diagnostic for predicting response to anti-TNF treatment in Crohn's disease. In patients with Crohn's disease not treated with anti-TNF therapy, live endoscopic imaging of gut mucosa with fluorescently labeled Humira adalimumab, an anti-TNF antibody, identified mTNF-expressing intestinal cells. High numbers of mTNF-expressing cells correlated with clinical response to subsequent anti-TNF antibody therapy, and low numbers of mTNF-expressing cells correlated with lack of clinical response. Next steps include comparable studies in ulcerative colitis and multicenter studies to test a larger population of patients with Crohn's disease. Humira is marketed by AbbVie Inc. to treat several autoimmune diseases. *SciBX* 7(13); doi:10.1038/scibx.2014.388 Published online April 3, 2014	Patent filed by the University of Erlangen-Nuremberg; unavailable for licensing	Atreya, R. *et al. Nat. Med.*; published online Feb. 23, 2014; doi:10.1038/nm.3462 **Contact:** Markus F. Neurath, University of Erlangen-Nuremberg, Erlangen, Germany e-mail: markus.neurath@uk-erlangen.de

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