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Germinating MALT1

By Chris Cain, Senior Writer

AstraZeneca plc has teamed up with the **Flanders Institute for Biotechnology** and the **Centre for Drug Design and Discovery** to make the first disclosed industry play for inhibitors of MALT1. The deal announcement caps five years of progress toward validating MALT1 in B cell lymphoma and autoimmune diseases.

MALT1 (mucosa associated lymphoid tissue lymphoma translocation gene 1) was first identified as a driver of a small subset of B cell lymphomas more than a decade ago. The protein was quickly implicated by **Roche's Genentech Inc.** unit and other groups as a regulator of B and T cell signaling and NF-κB activity,¹ but its precise molecular function and the potential druggability of the target remained unclear.

That changed in 2008 when two independent teams—one led by the **University of Lausanne**² and one led by Rudi Beyaert at the Flanders Institute for Biotechnology (VIB),³ reported in *Nature Immunology* that MALT1 functions as a protease through a conserved paracaspase domain.

Beyaert is associate department director of the VIB inflammation research center. He was joined on the paper by Thijs Baens, a staff scientist at VIB, and Peter Marynen, a professor of human molecular genetics at the **Catholic University Leuven**. In 2008, the team also filed a patent application describing peptide and small molecule inhibitors of MALT1.

Last week, AstraZeneca licensed a series of small molecule inhibitors from the team and partnered with them to further study MALT1 function.

AstraZeneca will make undisclosed upfront and milestone payments to VIB and the Centre for Drug Design and Discovery (CD3), an organization that performs drug discovery for academic laboratories and small, regional biotechs. The partners also are eligible for royalties.

"The work on building a translation package for this target will be jointly carried out in the labs of VIB/CD3 and AstraZeneca in Moelndal, Sweden. The work on the compounds will be carried out mainly at AstraZeneca in Moelndal," said Stefaan Allemeersch, director of business development at CD3.

"MALT1 is the only protease of its kind in the human genome, and the literature confirms that allosteric modes of action may be of particular interest."

—Jodi Lewis,
AstraZeneca plc

Fermenting strength

In the last five years, a series of key studies—most of which were featured in *SciBX*—strengthened the therapeutic rationale of targeting MALT1 in

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B cell lymphoma and broadened the potential applications of inhibiting MALT1 to autoimmune disease.

For example, a 2009 paper by a University of Lausanne and **National Cancer Institute** team reported that a peptide inhibitor was selectively toxic to activated B cell-like diffuse large B cell lymphoma (ABC-DLBCL) cells.⁴ Separate work published by the **Technical University Munich** and the **German Research Center for Environmental Health** came to a similar conclusion.⁵

In 2011, a **University of Michigan** team published a precise molecular explanation of how a MALT1 fusion oncoprotein drives the MALT lymphoma subtype by cleaving MAP kinase kinase kinase 14 (MAP3K14; NIK).⁶

In 2012, two teams simultaneously published *in vivo* data showing the potential of small molecule MALT1 inhibitors.

A team led by Ari Melnick, a professor of medicine at **Weill Cornell Medical College**, showed that nanomolar-potent MALT1 inhibitors had efficacy in a mouse model of ABC-DLBCL.⁷ Melnick told *SciBX* last week that the licensing status of his team's series of MALT1 inhibitors is undisclosed.

The other team was led by Daniel Krappmann, head of the research unit for cellular signal integration at the Institute of Molecular Toxicology and Pharmacology at the German Research Center for Environmental Health. His team showed that the antipsychotic compounds mepazine and thioridazine were also nanomolar-potent MALT1 inhibitors with *in vivo* efficacy in ABC-DLBCL.⁸

Krappmann told *SciBX* that his team has since synthesized new inhibitors but also sees potential for thioridazine in the clinic. "We are in the process of working out a clinical repurposing trial with thioridazine because this drug is still available. We are currently still profiling our

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“I think the announcement of the MALT1 discovery partnership between AstraZeneca and VIB underscores clearly that MALT1 is a very attractive target for autoimmune diseases and distinct types of cancers.”

**—Daniel Krappmann,
German Research Center for
Environmental Health**

new MALT1 inhibitors, and they are still available for licensing,” he said.

His lab has filed for four patents covering discoveries relating to MALT1.

Most recently, at least two studies have suggested that inhibiting MALT1 could help treat autoimmune disease.

In late 2012, a team from the **University of Toronto** led by Tak Mak showed that *Malt1* knockout prevented mice from developing experimental autoimmune

encephalomyelitis (EAE) by suppressing the differentiation of functional T helper type 17 (Th17) cells.⁹ Mak is a professor at the university and director of **The Campbell Family Institute for Breast Cancer Research at The Princess Margaret**.

In early 2013, a VIB team including Beyaert published similar results.¹⁰ Jérôme Van Biervliet, senior business development manager at VIB, credited recent publications from VIB and other academic teams for functionally validating MALT1 and showing its druggability.

Both Krappmann and Melnick said that some of their next steps include looking at the role of MALT1 in autoimmunity.

Krappmann’s MALT1 inhibitors act through an allosteric mechanism.¹¹ AstraZeneca spokesperson Jodi Lewis did not disclose the mechanism of action for the VIB/CD3 compounds licensed by AstraZeneca but added that specificity for the target, often a problem for protease inhibitors, was not a concern.

“MALT1 is the only protease of its kind in the human genome, and the literature confirms that allosteric modes of action may be of particular interest,” she said.

Although Genentech scientists have written at least two recent news pieces for scientific journals highlighting publications about MALT1, they have not published on inhibitors of the target, and no MALT1 inhibitors

have been disclosed in patents or publications from other industry teams.

Lewis said that there has been increasing interest in MALT1, and Krappmann added that his lab has been in contact with undisclosed pharmaceutical companies. “I think the announcement of the MALT1 discovery partnership between AstraZeneca and VIB underscores clearly that MALT1 is a very attractive target for autoimmune diseases and distinct types of cancers,” he said.

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PCSK9 peptide inhibitors

By Lauren Martz, Staff Writer

Although at least 10 companies are chasing inhibitors of PCSK9 for decreasing low-density lipoprotein cholesterol, the compounds dominating the field are antibodies or siRNAs that require injection. Roche's Genentech Inc. unit and a separate team from Pfizer Inc. and The University of Queensland are betting that peptides might provide an oral competitor and have identified short peptide fragments that inhibit binding of PCSK9 to its target.^{1,2} Optimizing the potency and stability for oral delivery is the next challenge.

Genentech is not disclosing plans for future development of the peptides. Pfizer did not reply to enquiries regarding future plans for its compounds.

The low-density lipoprotein receptor (LDLR) decreases circulating LDL cholesterol by binding the lipid at the hepatocyte cell surface, causing internalization of the LDL-LDLR complex. Inside the cells, LDL dissociates from LDLR and recycles to the cell surface, whereas the lipid is degraded by the lysosome.

PCSK9 (proprotein convertase subtilisin/kexin type 9) is a key regulator of LDL that acts by decreasing surface levels of LDLR, which reduces uptake of the lipid from the blood.

Figure 1. PCSK9-mediated regulation of LDLR levels. Proprotein convertase subtilisin kexin type 9 (PCSK9) is highly expressed in the liver and is a validated target for hypercholesterolemia. In *Chemistry & Biology*, researchers showed that truncated peptide analogs of the epidermal growth factor-like A domain (EGF-A) of the low-density lipoprotein receptor (LDLR) block receptor interaction with PCSK9 and may reduce circulating LDL cholesterol.²

[a] PCSK9 is a protease that is synthesized as an enzyme precursor. Following synthesis, PCSK9 undergoes autocatalytic cleavage, which is required for secretion from the cell. Although cleaved, the two domains of PCSK9 remain together.

[b(1)] When LDL cholesterol binds to LDLR, the receptor ligand is internalized, thus removing LDL cholesterol from circulation. When LDL binds a PCSK9-bound LDLR, the entire complex is internalized. PCSK9 then directs the LDLR to the lysosome, where it is degraded and prevented from recycling to the cell surface. The short peptides block the interaction between PCSK9 and its EGF-A-binding site on LDLR.

[b(2)] If an internalized LDLR is not bound to PCSK9, the receptor is recycled to the cell surface, where it continues to remove LDL cholesterol from circulation.

A number of companies are developing compounds that decrease PCSK9 activity, blocking synthesis of the protein with RNA-targeting therapeutics, mAbs or small molecules that inhibit PCSK9 binding to LDLR.

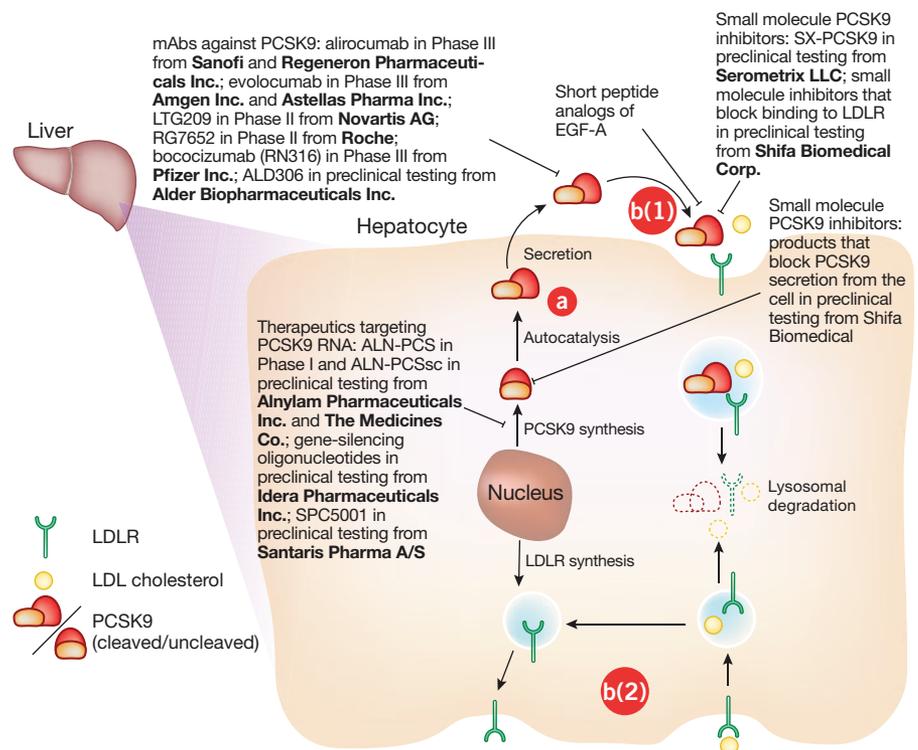
PCSK9 is synthesized as an inactive proenzyme and undergoes autocatalysis to form the active protein that binds LDLR. When the PCSK9-bound LDLR binds LDL, the whole complex is internalized. PCSK9 then directs the bound LDLR to the lysosome, where it is degraded and prevented from recycling to the cell surface (see **Figure 1**, "PCSK9-mediated regulation of LDLR levels").

The protein has thus become the focus of intense activity as a target for lowering circulating LDL by blocking the interaction between PCSK9 and LDLR.

Several PCSK9-targeted antibodies and siRNA candidates are in clinical and preclinical development for various dyslipidemias (see **Figure 1**, "PCSK9-mediated regulation of LDLR levels"). However, these compounds all require parenteral administration, and orally available inhibitors still lag far behind.

Serometrix LLC and Shifa Biomedical Corp. have disclosed preclinical small molecule programs for oral inhibitors of PCSK9. Serometrix is developing allosteric ligands of PCSK9 that disrupt normal protein folding to inhibit LDLR binding. Shifa is developing small molecules that block the autocatalytic cleavage of PCSK9 to prevent secretion from the cell and small molecules that block the interaction between PCSK9 and LDLR.

In general, protein-protein interactions do not lend themselves to small molecule inhibition. 3D crystallography shows that the site of interaction between PCSK9 and LDLR is relatively flat and does not have pockets that enable small molecule binding.³



Now, two separate teams have taken different approaches to exploring whether peptides could block the binding interaction better than small molecules, offering a potential alternative for creating oral inhibitors of PCSK9.

Short peptide inhibitors

David Craik and colleagues used rational drug design to create peptides that would competitively inhibit the interaction between PCSK9 and its binding site on LDLR, which lies in the receptor's epidermal growth factor-like A domain (EGF-A).²

Craik is laboratory head of the Chemistry and Structural Biology Division at the University of Queensland. The study also included researchers from Pfizer.

The team tested various truncated analogs of EGF-A and found a 26-amino-acid peptide analog lacking the C-terminal region that bound PCSK9.

To optimize the peptide's binding affinity, they introduced a gain-of-function mutation associated with genetic predisposition to hypercholesterolemia that enhances the binding of LDLR to PCSK9.

In binding assays, the modified analog bound PCSK9 with a K_d of $\sim 0.6 \mu\text{M}$, which was about twofold more potent than binding for full-length EGF-A. The peptide also competitively inhibited binding of PCSK9 to LDLR in *in vitro* assays and promoted recycling of LDLR to the cell surface in PCSK9-treated hepatocarcinoma cells. In the latter two assays, the peptide's potency was about five- to sevenfold lower than that of full-length EGF-A.

Results were published in *Chemistry & Biology*.

In the Genentech study, the team screened phage-display libraries for peptides that bound PCSK9 and then optimized the highest-affinity peptides using SAR studies.¹

The most potent peptide was a 13-amino-acid mimetic of EGF-A that had structural features comparable to those of the full-length EGF-A and bound PCSK9 via similar intermolecular contacts.

In binding assays, the phage display-derived peptide bound PCSK9 with a K_d of $\sim 0.7 \mu\text{M}$. This peptide also restored LDL uptake in hepatocarcinoma cells pretreated with PCSK9.

Data were published in *The Journal of Biological Chemistry*.

There is no overlap between the peptides identified in the two papers.

Peptide hurdles

Craik told *SciBX* that the next steps for his team include improving the potency and biopharmaceutical properties of the peptides. His team has not yet tested the peptides in animal models.

Converting peptides to oral therapeutics has proved difficult in many therapeutic areas.

Roger Newton, executive chairman and CSO at **Esperion Therapeutics Inc.**, said, "The biggest challenge with peptides is achieving oral delivery, which requires high stability, small size and potency. Unless the peptides are modified for protection, they will be proteolytically degraded in the stomach and intestines before they even have a chance at a therapeutic benefit."

Esperion has ETC-1002, a small molecule ATP citrate lyase (ACLY) inhibitor and AMP-activated protein kinase (AMPK) activator, in Phase IIB testing to treat hypercholesterolemia.

Serometrix CEO Michael Muehleemann said that one way to avoid the problems with peptide drug delivery is to convert small peptides into small molecules. However, he does not expect this strategy will work for the EGF-A peptides.

He told *SciBX* that his team looked at competitive inhibitors of EGF-A binding early in the PCSK9 program. Although the researchers identified some peptides that blocked binding, the activity decreased as they reduced peptide size to enable oral delivery.

Muehleemann added that based on the size and relatively flat nature of the orthosteric EGF-A site, there would be a high risk in trying to develop peptides small enough for conversion to small molecules.

Newton added, "The work provides great proof of concept, but this work is still *in vitro*. Taking the peptide therapeutics to the whole-animal level and proving efficacy is the next step and a big challenge."

He noted, "The take-home message is that it is possible to disrupt the interaction with small peptides, which is a significant advancement. However, the amount of optimization that the teams went through in these papers speaks to how hard it would be to develop an effective oral peptide."

If the teams can overcome the hurdles associated with designing oral peptides, the peptides could have benefits over existing cholesterol-lowering drugs and PCSK9 mAbs.

"There is a big market for statin alternatives. Two to seven million patients in America are intolerant of statins due to side effects such as muscle pain," said Newton. "Everyone in the field is looking for a compound that is an improvement over statins."

Craik did not disclose the patent status of his work. The work was sponsored by and licensed to Pfizer.

The patent and licensing status of the peptides from the *JBC* paper is unavailable.

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COMPANIES AND INSTITUTIONS MENTIONED

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"The work provides great proof of concept, but this work is still *in vitro*. Taking the peptide therapeutics to the whole-animal level and proving efficacy is the next step and a big challenge."

**—Roger Newton,
Esperion Therapeutics Inc.**

Negative nanoparticles

By C. Simone Fishburn, Senior Editor

A simple experimental error has opened up the potential for nanoparticles to treat disease rather than be used only as payloads. By accidentally using negatively charged nanoparticles instead of neutral ones, a team from **Northwestern University** and **The University of Sydney** saw selective tagging of inflammatory monocytes for destruction.¹

Their spinout, **Cour Pharmaceutical Development Co. Inc.**, is testing the particles in myocardial infarction (MI) and plans to enter Phase I testing within a year.

Inflammatory monocytes (IMs) are a subset of immature immune cells that cause tissue damage in MI and a range of other immune-mediated diseases when they rapidly infiltrate inflamed tissue in response to an injury and differentiate into macrophages or dendrocytes.

The result is the release of proinflammatory cytokines, proteases and toxic agents such as nitric oxide.

Most attempts to dampen the inflammatory response focus on inhibiting targets such as proinflammatory cytokines. This can lead to widespread immune suppression caused by the ubiquitous expression of the cytokines. By contrast, the researchers' negative nanoparticle approach aims to neutralize IMs, which lie at the root of the damage.

Accentuate the negative

Daniel Getts, Nicholas King and Stephen Miller previously collaborated on a study of the ability of antigen-conjugated nanoparticles to induce immune tolerance.^{2,3} This time they teamed up to investigate how immune cells enter the inflamed brain and used nanoparticles to track the cells' movement.

Getts is CSO at Cour. King is a professor of viral immunopathology at the **Sydney Medical School at The University of Sydney**. Miller is a professor of microbiology-immunology and dermatology at the **Northwestern University Feinberg School of Medicine**.

The researchers used a mouse model of West Nile virus encephalitis to produce neuroinflammation and inadvertently gave the animals carboxylated polystyrene particles, which are negatively charged, instead of uncoated, neutral ones.

The researchers used a mouse model of West Nile virus encephalitis to produce neuroinflammation and inadvertently gave the animals carboxylated polystyrene particles, which are negatively charged, instead of uncoated, neutral ones.

More than 60% of the infected animals survived, which was in stark contrast to the 100% lethality seen in all their previous experiments using neutral particles.

"There is no antigen, no ligand, no immune-suppressing drug. We actually found a therapeutic utility for a pure particle with no attachment," Getts told *SciBX*.

"There is no antigen, no ligand, no immune-suppressing drug. We actually found a therapeutic utility for a pure particle with no attachment."

— Daniel Getts,
Cour Pharmaceutical Development Co. Inc.

The negative charge was the dominant factor in protecting the animals. Positively charged particles produced some toxicity, whereas neutral particles showed no efficacy, King told *SciBX*.

According to Getts, the key was to fine-tune the charge on the particles to a ζ -potential of about -50 mV. ζ -Potential is a measure of the surface charge used in nanoparticle characterization.

Nanoparticle-treated animals that survived showed far fewer IM-derived macrophages in the brain than animals treated with neutral particles or vehicle.

The next step was determining how the nanoparticles prevented IMs from reaching the brain. The group tracked the path of IMs in nanoparticle-treated animals and learned that a membrane-bound scavenger protein, macrophage receptor with collagenous structure (MARCO; Scara2), on IMs redirects them to the spleen, where they undergo apoptosis and are eliminated.

Diversion to the spleen prevented the IMs from reaching the site of inflammation and causing damage. In splenectomized animals, the nanoparticles had no protective effect and IM numbers in the brain were comparable to those in untreated mice.

Although the team is continuing to explore precisely how the nanoparticles interact with MARCO, the electrostatic interaction is most likely central to the process, Miller said. MARCO contains a positively charged collagen domain and is known to bind to polyanionic surfaces.

Getts added that MARCO is the protein the team identified in mice, but there is a range of other scavenger receptors that might also be involved in the nanoparticle interaction. Thus, he said, an antibody or small molecule against MARCO probably would not be as effective as using the whole nanoparticle.

The nanoparticles improved disease outcomes in models of immune-mediated conditions including MI, peritoneal inflammation, multiple sclerosis (MS), inflammatory bowel disease (IBD) and cardiac and kidney reperfusion injury.

For example, in MI the negative nanoparticles increased the heart's ejection fraction and the percentage fraction shortening compared with vehicle.

Results were published in *Science Translational Medicine*.

Taking the IMs out of MI

Miller said that the negative nanoparticles might offer a way to limit damage when patients with MI arrive at the hospital. "Limiting early inflammatory migration when heart cells are dying could change the course of the disease," he said.

In MI, much of the damage to myocardial tissue is caused by the initial influx of IMs to the site of infarction.

According to Matthias Nahrendorf, the use of nanoparticles to take IMs out of the picture could be a good step forward.

"Traditionally, cardiologists thought like plumbers and would focus on the coronary artery, but we are very excited that people are finally thinking about the inflammatory response in MI," he said.

Nahrendorf is an associate professor of radiology at **Massachusetts General Hospital** and works on molecular processes of healing in MI.

"Limiting early inflammatory migration when heart cells are dying could change the course of the disease."

— Stephen Miller,
Northwestern University Feinberg School of Medicine

Although multiple groups have tried to block migration of IMs by targeting chemokine receptors,⁴⁻⁶ neither Getts nor Nahrendorf were aware of any other companies targeting IMs directly.

Nahrendorf said that finding the right dose of nanoparticles might be the biggest challenge. Too many nanoparticles could overly deplete IMs and lead to immune suppression. Too few would have muted therapeutic effects. In addition, he said, the team should determine whether—and at what doses—the nanoparticles bind other phagocytic cells.

Getts, King and coauthor Rachael Terry, a postdoctoral research associate at the Feinberg School of Medicine, have filed for a patent covering the findings, and the IP is licensed to Cour.

Cour CEO John Puisis told *SciBX* that acute MI is the first indication the company will pursue. Cour is running IND-enabling studies in Australia and hopes to start Phase I studies within 12 months.

The company also is working with groups in Nepal and other developing countries to use the technology in flavivirus infections similar to West Nile virus.

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COMPANIES AND INSTITUTIONS MENTIONED

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RNA is for activation

By Amy Donner, Senior Editor

Although siRNA-based therapeutics have recently been clinically validated as a strategy to knock down gene expression, a lesser-known function for short double-stranded RNAs is their ability to turn on the expression of target genes. Now, an academic team sponsored by **MiNA Therapeutics Ltd.** has found a way to attack liver cancer in rats by using a short activating RNA to upregulate expression of a tumor suppressor,¹ and the company plans to advance the program to the clinic this year.

siRNAs target mRNA coding regions and are used to knock down gene expression via targeted cleavage by an RNA-induced silencing complex (RISC). The clinical development of this strategy was pioneered by **Alnylam Pharmaceuticals Inc.**, which last month entered into a major collaboration to develop products with the **Genzyme Corp.** unit of **Sanofi**.²

At least 12 additional companies are pursuing the development of RNAi-based therapeutics, one of which, **Dicerna Pharmaceuticals Inc.**, went public last week and saw its stock more than triple in its first day of trading. The preclinical-stage company ended the week with a market cap of \$683.7 million.

Small activating RNAs (saRNAs) are structurally related to siRNAs, but instead of targeting mRNA, they target regulatory regions of DNA upstream of genes of interest. This can lead to increased gene expression, though the precise mechanisms of action for saRNAs are poorly understood.

MiNA CEO Robert Habib told *SciBX*, “The beauty of saRNA is that well-established oligonucleotide designs can be used as triggers for gain of function. That allows MiNA to leverage years of pharmaceutical development, from assays to delivery, which have enabled a robust pipeline of ‘loss-of-function therapeutics.’”

These advances include the development of lipid nanoparticle formulations that enable efficient short double-stranded RNA delivery to the liver in humans.

Thus, MiNA set out to design a liver-targeted saRNA to treat hepatocellular carcinoma (HCC).

Prognosis for patients with HCC is poor because a majority have advanced disease at the time of diagnosis, which impairs liver function and prevents surgery. Thus, therapeutic strategies that antagonize tumor formation and improve or preserve liver function are needed.

The company homed in on CCAAT enhancer binding protein- α (CEBPA), a tumor suppressor that is also known to upregulate genes involved in hepatocyte function, such as albumin. *CEBPA* is expressed in functional hepatocytes in the liver but is downregulated in hepatoma cells.

“CEBPA is a transcription factor with a number of regulatory functions including controlling hepatocyte response to injury,” said Habib. “Evidence suggested that these features could be translated into a promising therapeutic candidate.”

John Rossi, cofounder of both Dicerna and MiNA, said that the target was attractive because it is a master regulator of gene expression for a large group of genes that alter cell proliferation.

Rossi is chair and professor of molecular and cellular biology and dean at **City of Hope** and is on the scientific advisory board for several companies developing RNA therapeutics including Dicerna, **Arrowhead Research Corp.** and **Benitec Biopharma Ltd.**

In a study led by Rossi and Nagy Habib—Robert Habib’s father—scientists tested whether an saRNA that upregulated *CEBPA* expression could be used to improve cirrhotic liver function, treat HCC or both.¹ Nagy Habib is lead clinician and head of the Department of Hepatobiliary Surgery at **Imperial College London**.

To develop an saRNA candidate, the team used bioinformatics approaches to search databases for antisense transcripts that overlapped with *CEBPA* and then used a design algorithm to predict short double-stranded RNAs that might act as saRNAs by blocking the antisense transcript.

In a cultured human liver carcinoma cell line, an saRNA targeting the *CEBPA* promoter region doubled *CEBPA* transcript levels, increased albumin secretion and decreased proliferation compared with scrambled saRNA. The *CEBPA*-saRNA also reduced DNA methylation near the *CEBPA* promoter, hinting at a possible molecular explanation for its activity.

To test the saRNA *in vivo*, the scientists conjugated it to a triethanolamine core poly(amidoamine) dendrimer that has previously been used to deliver siRNA to the liver *in vivo*.³

In a rat model of liver cirrhosis with HCC, the *CEBPA*-saRNA dendrimer increased expression of *Cebpa* and levels of

circulating albumin and decreased activity of the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) compared with scrambled saRNA dendrimer. Gene expression data were also consistent with improved liver function in *CEBPA*-saRNA dendrimer-treated rats.

The *CEBPA*-saRNA dendrimer also decreased tumor formation and led to a 10% decrease in the expression of a preneoplastic marker.

The study was published in *Hepatology* and included scientists from the **Norwegian University of Science and Technology**, **National Taiwan University**, **Marseille Interdisciplinary Center for Nanoscience**, **General University Hospital of Larissa**, **Biomedical Research Foundation of the Academy of Athens**, **University of California, Los Angeles**, **High Technology Medical Center**, **Tbilisi State Medical University**, **Qatar Biomedical Research Institute** and **University of Pennsylvania**. It was funded by MiNA.

The devil is in the details

Although researchers contacted by *SciBX* agreed that upregulating *CEBPA* holds promise in HCC, they were concerned by the lack of a clear molecular explanation for saRNA action.

Jim Barsoum, CSO of **RaNA Therapeutics Inc.**, said that there were many possible ways the saRNA could be acting. “These saRNAs could

“The beauty of saRNA is that well-established oligonucleotide designs can be used as triggers for gain of function. That allows MiNA to leverage years of pharmaceutical development, from assays to delivery, which have enabled a robust pipeline of loss-of-function therapeutics.”

—Robert Habib,
MiNA Therapeutics Ltd.

“We now are realizing that oligonucleotides are a rich and diverse therapeutic modality that can possess a wide variety of different activities.”

—Jim Barsoum,
RaNA Therapeutics Inc.

be blocking the activity or inducing the degradation of antisense transcripts that repress transcription. The saRNA may be modulating the recruitment of histone methyltransferases or demethylases that naturally lead to the repression of transcription. Some data suggest that an RNAi-mediated mechanism may be responsible,” he said.

RaNA is developing short single-stranded oligonucleotides to selectively activate target gene expression. One platform is based upon the ability of these oligonucleotides to sterically block the interactions between RNA and polycomb repressive complex 2 (PRC2).

“Current drug development strategies place great value on having a thorough mechanistic understanding,” added Barsoum.

Claes Wahlestedt agreed. “saRNAs are arguably controversial, and the underlying mechanism of CEBPA-saRNA is almost entirely unclear,” he said. He added that he would be more confident about the therapeutic strategy if the mechanism was better understood.

Wahlestedt is associate dean for therapeutic innovation, director of the Center for Therapeutic Innovation and a professor of psychiatry and behavioral sciences at the **University of Miami Miller School of Medicine**. He also cofounded Curna Inc. to commercialize a distinct single-stranded noncoding RNA-based strategy for upregulating gene expression. **Opko Health Inc.** acquired Curna in 2011.

MiNA acknowledges the need to clarify the mechanism. “The exact mechanism of transcriptional gene activation is still being defined,” said Robert Habib. He indicated that some general features about saRNA action have been established, and MiNA has evidence from chromatin immunoprecipitation assays that CEBPA-saRNA interacts specifically with the transcription start site of the *CEBPA* promoter.

Rossi added that a noncoding RNA is likely involved. “We believe that an saRNA–long noncoding RNA complex recruits chromatin-remodeling enzymes, resulting in positive histone marks and also recruiting RNA polymerase to the targeted promoter.”

A noncoding RNA-mediated mechanism for regulating *CEBPA* expression was published last year in *Nature* by an independent group from **Harvard Medical School**.⁴ The scientists reported a noncoding RNA—called *ecCEBPA*—derived from *CEBPA* that binds DNA (cytosine-5-methyltransferase 1 (DNMT1), preventing methylation of the gene and increasing expression of *CEBPA*. MiNA is investigating the relationship between *ecCEBPA* and their CEBPA-saRNA.

A clinical formulation

MiNA has now begun clinical testing of a nanoparticle-based formulation of a *CEBPA*-upregulating saRNA, MTL-501. “MTL-501 is in a first-in-human dose-escalation study,” said Robert Habib. MiNA anticipates initiating a Phase I study in the U.K. in under a year.

MTL-501 is an optimized version of the published CEBPA-saRNA dendrimer.

Barsoum and Wahlestedt both noted that formulating and manufacturing CEBPA-saRNA is not trivial because double-stranded RNA is more difficult to deliver than single-stranded RNA.

“Although other published studies have shown this type of nanoparticle

delivery vehicle to be safe, the complex creates manufacturing challenges and could lead to its own set of toxicities,” said Barsoum.

“Using dendrimers for delivery is not uncomplicated,” added Wahlestedt.

However, Rossi told *SciBX* that MiNA is moving forward to ramp up production. “We know from our preclinical animal work that this is a safe and efficacious approach for treating liver cancer and fatty liver disease, so we would like to scale up manufacturing of the components for a Phase I human trial,” he said.

Robert Habib said that the CEBPA-saRNA has been tested in a range of subcutaneous tumor xenograft and fatty liver disease models in addition to the published work in the rat model of cirrhosis with HCC.

The product and its method of use are covered by MiNA’s patent portfolio, and MTL-501 is available for partnering. MiNA is also developing additional saRNA therapeutic candidates. “Now that loss-of-function oligonucleotide-based strategies have been substantially derisked, gain of function is a strikingly obvious next step,” Robert Habib said.

Barsoum agreed. “For many years, oligonucleotides have been viewed as a means to decrease gene expression. There are now multiple examples of oligonucleotides being used to increase gene expression,” he said. “We now are realizing that oligonucleotides are a rich and diverse therapeutic modality that can possess a wide variety of different activities.”

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2. McCallister, E. *BioCentury* 22(3), A1–A5; Jan. 20, 2014
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COMPANIES AND INSTITUTIONS MENTIONED

Anlyam Pharmaceuticals Inc. (NASDAQ:ALNY), Cambridge, Mass.
Arrowhead Research Corp. (NASDAQ:ARWR), Pasadena, Calif.
Benitec Biopharma Ltd. (ASX:BLT), Balmain, New South Wales, Australia
Biomedical Research Foundation of the Academy of Athens, Athens, Greece
City of Hope, Duarte, Calif.
Dicerna Pharmaceuticals Inc. (NASDAQ:DRNA), Watertown, Mass.
General University Hospital of Larissa, Larissa, Greece
Genzyme Corp., Cambridge, Mass.
Harvard Medical School, Boston, Mass.
High Technology Medical Center, Tbilisi, Georgia
Imperial College London, London, U.K.
Marseille Interdisciplinary Center for Nanoscience, Marseille, France
MiNA Therapeutics Ltd., London, U.K.
National Taiwan University, Taipei City, Taiwan
Norwegian University of Science and Technology, Trondheim, Norway
Opko Health Inc. (NYSE:OPK; Tel Aviv:OPK), Miami, Fla.
Qatar Biomedical Research Institute, Doha, Qatar
RaNA Therapeutics Inc., Cambridge, Mass.
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
Tbilisi State Medical University, Tbilisi, Georgia
University of California, Los Angeles, Calif.
University of Miami Miller School of Medicine, Miami, Fla.
University of Pennsylvania, Philadelphia, Pa.

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
Autoimmune disease				
Rheumatoid arthritis (RA)	Cyclin dependent kinase 4 (CDK4); CDK6	Human genetic studies suggest antagonizing CDK4 or CDK6 activity could be useful for treating RA. A meta-analysis of previously reported genome-wide association and mouse knockout studies identified polymorphisms in 98 genes that contributed to RA risk, including 27 genes that encode proteins that are targets of approved RA drugs. Two risk genes— <i>CDK4</i> and <i>CDK6</i> —encode proteins that are targets of at least three compounds in clinical testing for a range of cancers. Next steps include testing CDK4 and CDK6 inhibitors in models of RA. Dual CDK4 and CDK6 antagonists in clinical testing for cancer include Pfizer Inc.'s Phase III compound palbociclib (PD-0332991), Novartis AG and Otsuka Pharmaceutical Co. Ltd.'s Phase III compound LEE011 and Eli Lilly and Co.'s Phase II compound LY2835219. SciBX 7(5); doi:10.1038/scibx.2014.137 Published online Feb. 6, 2014	Patent and licensing status not applicable	Okada, Y. <i>et al. Nature</i> ; published online Dec. 25, 2013; doi:10.1038/nature12873 Contact: Robert M. Plenge, Harvard Medical School, Boston, Mass. e-mail: robert.plenge@merck.com
Cancer				
Breast cancer	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily c member 1 (SMARCC1; BAF155); coactivator-associated arginine methyltransferase 1 (CARM1)	<i>In vitro</i> and mouse studies suggest inhibiting BAF155 methylation by CARM1 could help treat metastatic breast cancer. In breast cancer cells, BAF155 was identified as a substrate of the CARM1 methyltransferase. In the cells, <i>BAF155</i> knockout decreased cell growth and migration, which was restored by expression of wild-type <i>BAF155</i> but not of a variant with a mutated methylation site. In tissue samples from breast cancer patients, lack of BAF155 methylation increased recurrence-free survival compared with the presence of BAF155 methylation. In mouse xenograft models of metastatic breast cancer, expression of a methylation-resistant <i>BAF155</i> mutant prevented tumor growth in the lungs. Next steps include identifying inhibitors of CARM1-mediated BAF155 methylation. SciBX 7(5); doi:10.1038/scibx.2014.138 Published online Feb. 6, 2014	Unpatented; licensing status not applicable	Wang, L. <i>et al. Cancer Cell</i> ; published online Jan. 13, 2014; doi:10.1016/j.ccr.2013.12.007 Contact: Wei Xu, University of Wisconsin–Madison, Madison, Wis. e-mail: wxu@oncology.wisc.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	Lysine-specific demethylase 4 (KDM4; JMJD2); lysine-specific demethylase 1A (KDM1A; LSD1)	<p>SAR studies suggest compounds that inhibit both LSD1 and JMJD2 could be useful for treating cancer. <i>In vitro</i>, coupling known LSD1 and JMJD2 inhibitors led to the creation of a compound that had a submicromolar IC₅₀ value for LSD1 and an IC₅₀ of ~1.2 μM for JMJD2. In three cancer cell lines, the compound increased histone methylation and apoptosis compared with parent compounds given alone but did not induce apoptosis in noncancerous mesenchymal progenitor cells. Next steps could include preclinical testing and optimization of dual inhibitors of the targets.</p> <p>Oryzon Genomics S.A. has the LSD1 inhibitor ORY-1001 in Phase I/IIa trials for acute myelogenous leukemia (AML).</p> <p>Salarius Pharmaceuticals LLC's SP-2528, a selective LSD1 inhibitor, is in preclinical development for cancer.</p> <p>GlaxoSmithKline plc has LSD1 inhibitors in preclinical development to treat AML.</p> <p>SciBX 7(5); doi:10.1038/scibx.2014.139 Published online Feb. 6, 2014</p>	Patent and licensing status undisclosed	<p>Rotili, D. <i>et al. J. Med. Chem.</i>; published online Dec. 10, 2013; doi:10.1021/jm4012802</p> <p>Contact: Antonello Mai, Sapienza University of Rome, Rome, Italy e-mail: antonello.mai@uniroma1.it</p>
Cancer	Protein phosphatase magnesium-dependent 1δ (PPM1D; WIP1)	<p><i>In vitro</i> and mouse studies identified an allosteric inhibitor of WIP1 that could help treat cancer. WIP1 is overexpressed in some cancers and dephosphorylates tumor suppressor genes to block their activity. In multiple cancer cell lines expressing WIP1, a WIP1 inhibitor that bound a specific subdomain near the catalytic site increased phosphorylation of p53 and other tumor suppressor genes and decreased cell growth compared with vehicle. In mice with B cell lymphoma xenografts, the allosteric inhibitor decreased tumor growth compared with vehicle. Next steps include optimization of the inhibitor's pharmaceutical properties for clinical evaluation.</p> <p>SciBX 7(5); doi:10.1038/scibx.2014.140 Published online Feb. 6, 2014</p>	Patent application filed by GlaxoSmithKline plc; unavailable for licensing	<p>Gilmartin, A.G. <i>et al. Nat. Chem. Biol.</i>; published online Jan. 5, 2014; doi:10.1038/nchembio.1427</p> <p>Contact: Rakesh Kumar, GlaxoSmithKline plc, Collegeville, Pa. e-mail: rakesh.2.kumar@gsk.com</p> <p>Contact: Dirk A. Heerding, same affiliation as above e-mail: dirk.a.heerding@gsk.com</p>
Melanoma	G protein-coupled receptor 56 (GPR56)	<p>Mouse studies suggest stimulating GPR56 activity could help reduce melanoma growth by preventing the deposition of excess extracellular matrix. In xenograft mice, knockdown of <i>GPR56</i> in transplanted melanoma cells increased tumor growth compared with no alteration, but this effect was not seen in mice lacking <i>transglutaminase 2</i> (<i>Tgm2</i>; <i>Tg2</i>). In a xenograft mouse model of melanoma, overexpression of <i>GPR56</i> decreased extracellular deposition of Tg2 and fibronectin by driving GPR56 to bind to Tg2 and promote its endocytosis. Next steps include performing chemical screens for small molecules that can facilitate internalization of TG2 by GPR56.</p> <p>SciBX 7(5); doi:10.1038/scibx.2014.141 Published online Feb. 6, 2014</p>	Unpatented; licensing status not applicable	<p>Yang, L. <i>et al. Cancer Res.</i>; published online Dec. 19, 2013; doi:10.1158/0008-5472.CAN-13-1268</p> <p>Contact: Lei Xu, University of Rochester, Rochester, N.Y. e-mail: lei_xu@urmc.rochester.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Prostate cancer	γ -Secretase	<p>Cell culture and mouse studies suggest γ-secretase inhibitors could help treat prostate cancer. Presenilin 1 (PSEN1; PS1) is the catalytic subunit of γ-secretase. In a human prostate cancer cell line, siRNA knockdown of PS1 decreased production of the intracellular domain (ICD) of transforming growth factor-β receptor 1 (TGFBR1; ALK5) compared with no alteration. In various human cancer cell lines and in a mouse xenograft model of prostate cancer, a γ-secretase inhibitor decreased ICD formation, expression of proinvasive genes, the number of invasive cells and tumor volume compared with vehicle. Next steps include testing γ-secretase inhibitors in <i>in vivo</i> models of metastasis and measuring TGFBR1 ICD levels in samples from patients with prostate cancer.</p> <p>At least 10 companies have γ-secretase inhibitors in Phase II or earlier testing to treat Alzheimer's disease (AD) or cancer.</p> <p>SciBX 7(5); doi:10.1038/scibx.2014.142 Published online Feb. 6, 2014</p>	Patented; available for licensing from the Ludwig Institute for Cancer Research Ltd.	Gudey, S.K. <i>et al. Sci. Signal.</i> ; published online Jan. 7, 2014; doi:10.1126/scisignal.2004207 Contact: Marene Landström, Umeå University, Umeå, Sweden e-mail: marene.landstrom@medbio.umu.se

Cardiovascular disease

Hypercholesterolemia	Proprotein convertase subtilisin/kexin type 9 (PCSK9); low-density lipoprotein receptor (LDLR)	<p><i>In vitro</i> studies identified truncated analogs of the epidermal growth factor-like domain A (EGF-A) of LDLR that could aid the design of oral therapeutics to lower cholesterol. PCSK9 binds EGF-A on LDLR, inhibits receptor recycling and thus reduces removal of LDL from the blood. In binding assays, a truncated analog of EGF-A bound PCSK9 with a K_d of ~ 0.6 μM and inhibited its interaction with EGF-A with an IC_{50} of ~ 18 μM. In hepatocellular carcinoma cells, the truncated analogs induced LDLR recycling with an EC_{50} of ~ 25 μM. This study was performed in collaboration with Pfizer Inc., whose next steps include improving potency and biopharmaceutical properties.</p> <p>Amgen Inc.'s evolocumab, Regeneron Pharmaceuticals Inc. and Sanofi's alirocumab and Pfizer's bococizumab are mAbs targeting PCSK9, and all are in Phase III testing to treat lipid disorders.</p> <p>At least seven additional companies have PCSK9 inhibitors in clinical or preclinical development (<i>see PCSK9 peptide inhibitors, page 4</i>).</p> <p>SciBX 7(5); doi:10.1038/scibx.2014.143 Published online Feb. 6, 2014</p>	Patent status undisclosed; licensed to Pfizer; unavailable for licensing	Schroeder, C.I. <i>et al. Chem. Biol.</i> ; published online Jan. 16, 2014; doi:10.1016/j.chembiol.2013.11.014 Contact: David J. Craik, The University of Queensland, Brisbane, Queensland, Australia e-mail: d.craik@imb.uq.edu.au
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This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Dermatology				
Dermatitis	Signal transducer and activator of transcription 5 (STAT5); Janus kinase-2 (JAK-2)	Human tissue and mouse studies suggest inhibiting STAT5 could help treat atopic dermatitis. In patient tissue samples, numbers of mast cells with activated STAT5 were higher in skin lesions than in normal skin. In mouse models of atopic dermatitis, mast cell-specific deletion of <i>Stat5</i> decreased disease severity compared with <i>Stat5</i> expression. Also in the models, a JAK-2 inhibitor that blocked Stat5 activation decreased both levels of infiltrating mast cells into skin lesions and disease severity compared with vehicle. Planned work includes additional testing of JAK-2 inhibitors in the models. Jakafi, a JAK-1 and JAK-2 inhibitor from Incyte Corp. and Novartis AG, is approved to treat myeloproliferative disorder. At least 10 companies have JAK-2 inhibitors in preclinical or clinical development for multiple indications.	Unpatented; unlicensed; available for partnering	Ando, T. <i>et al. Cell Rep.</i> ; published online Jan. 9, 2014; doi:10.1016/j.celrep.2013.12.029 Contact: Toshiaki Kawakami, La Jolla Institute for Allergy & Immunology, La Jolla, Calif. e-mail: toshi@liji.org
SciBX 7(5); doi:10.1038/scibx.2014.144 Published online Feb. 6, 2014				
Wounds	O-linked N-acetylglucosamine (GlcNAc) transferase (OGT)	<i>In vitro</i> studies suggest blocking OGT activity could help treat diabetic skin wounds. In human keratinocytes cultured in hyperglycemic conditions, wound closure was delayed and general levels of O-GlcNAc addition to proteins was greater than that in cultures without excess glucose. In human keratinocytes cultured in hyperglycemic conditions, siRNA targeting OGT, the enzyme that adds O-GlcNAc to intracellular proteins, accelerated wound closure and decreased O-GlcNAc modifications compared with scrambled siRNA. Next steps include developing topical formulations of antisense oligonucleotides targeting OGT to treat diabetic wounds.	Patent application filed; available for licensing	Runager, K. <i>et al. J. Biol. Chem.</i> ; published online Jan. 7, 2014; doi:10.1074/jbc.M113.513952 Contact: David S. Rubenstein, The University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, N.C. e-mail: druben@med.unc.edu
SciBX 7(5); doi:10.1038/scibx.2014.145 Published online Feb. 6, 2014				
Endocrine/metabolic disease				
Gaucher's disease	Receptor-interacting serine-threonine kinase 3 (RIPK3; RIP3)	Mouse studies suggest inhibiting RIPK3 could help treat forms of Gaucher's disease with neurological symptoms. Enzyme replacement therapy to restore glucocerebrosidase (GCase; GCase) does not treat neurological symptoms of the disease caused by neuron loss. In a genetic mouse model of Gaucher's disease with neurological symptoms, compared with littermates without the disease, Ripk3, which is involved in programmed necrosis, was upregulated in brains. In a mouse model of Gaucher's disease induced by an irreversible GCase inhibitor, <i>Ripk3</i> knockout extended lifespan and improved motor coordination, and it decreased neuron loss compared with wild-type <i>Ripk3</i> expression. Next steps include developing blood brain barrier-penetrant small molecule inhibitors of the RIPK3 pathway.	Provisional patent application filed in the U.S.; available for licensing	Vitner, E.B. <i>et al. Nat. Med.</i> ; published online Jan. 19, 2014; doi:10.1038/nm.3449 Contact: Anthony H. Futerman, Weizmann Institute of Science, Rehovot, Israel e-mail: tony.futerman@weizmann.ac.il
SciBX 7(5); doi:10.1038/scibx.2014.146 Published online Feb. 6, 2014				

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Infectious disease				
Tuberculosis	<i>Mycobacterium tuberculosis</i> membrane protein MmpS4 (mmpS4); mmpS5	<i>In vitro</i> studies suggest inhibiting siderophore secretion in <i>M. tuberculosis</i> could help treat tuberculosis. MmpS4 and mmpS5 are required for <i>M. tuberculosis</i> siderophore export, which facilitates iron scavenging. In <i>M. tuberculosis</i> , mutant mmpS4 and mmpS5 led to defective siderophore export, iron uptake and cell growth. In <i>M. tuberculosis</i> with the mutant membrane proteins, compared with <i>M. tuberculosis</i> with wild-type membrane proteins, exogenous siderophores did not restore cell growth and led to increased cell death. Next steps could include identifying points of therapeutic intervention in the siderophore pathway. SciBX 7(5); doi:10.1038/scibx.2014.147 Published online Feb. 6, 2014	Findings unpatented; licensing status not applicable	Jones, C.M. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Jan. 13, 2013; doi:10.1073/pnas.1311402111 Contact: Michael Niederweis, The University of Alabama at Birmingham, Birmingham, Ala. e-mail: mnieder@uab.edu
Inflammation				
Inflammatory disease	Inflammatory monocytes	Studies in mice suggest negatively charged nanoparticles could help treat inflammatory diseases by preventing migration of inflammatory monocytes. In a mouse model of West Nile virus encephalitis, negatively charged nanoparticles decreased seizures and levels of inflammatory cytokines and chemokines and increased survival compared with neutral nanoparticles or vehicle. In mouse models of peritoneal inflammation, multiple sclerosis (MS), myocardial infarction (MI) or ischemia/reperfusion injury, the negatively charged nanoparticles decreased both migration of inflammatory monocytes to sites of inflammation and disease symptoms compared with neutral nanoparticles or vehicle. Ongoing studies at Cour Pharmaceutical Development Co. Inc. include toxicity testing to support clinical trials in MI (<i>see Negative nanoparticles, page 6</i>). SciBX 7(5); doi:10.1038/scibx.2014.148 Published online Feb. 6, 2014	Patented by Getts Consulting and Project Management; licensed to Cour Pharmaceutical	Getts, D.R. <i>et al. Sci. Transl. Med.</i> ; published online Jan. 15, 2014; doi:10.1126/scitranslmed.3007563 Contact: Nicholas J.C. King, Sydney Medical School at The University of Sydney, Sydney, New South Wales, Australia e-mail: nickk@pathology.usyd.edu.au Contact: Stephen D. Miller, Northwestern University Feinberg School of Medicine, Elmhurst, Ill. e-mail: s-d-miller@northwestern.edu Contact: Daniel R. Getts, same affiliation as above e-mail: d-getts@northwestern.edu
Neurology				
Multiple sclerosis (MS)	Fas apoptotic inhibitory molecule 3 (FAIM3; TOSO)	Mouse studies suggest inhibiting the surface receptor TOSO on dendritic cells could help treat MS. In a mouse model of experimental autoimmune encephalomyelitis (EAE), knockout of Toso decreased leukocyte brain infiltration and increased numbers of immune-suppressive T helper cells compared with mice expressing normal Toso levels. In the model, a Toso-Fc fusion protein decreased disease severity and progression in mice that had been allowed to develop EAE prior to treatment. Next steps could include identifying a relevant TOSO ligand and developing a TOSO-directed therapeutic. SciBX 7(5); doi:10.1038/scibx.2014.149 Published online Feb. 6, 2014	Patent and licensing status unavailable	Brenner, D. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Jan. 7, 2014; doi:10.1073/pnas.1323166111 Contact: Tak W. Mak, University Health Network, Toronto, Ontario, Canada e-mail: tmak@uhnres.utoronto.ca

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Pulmonary disease				
Pulmonary fibrosis	IL-25 (IL-17E)	<p>Patient sample and mouse studies suggest inhibiting IL-25 could help treat pulmonary fibrosis. In a mouse model of <i>Schistosoma mansoni</i> egg-induced pulmonary fibrosis, IL-25 knockout mice had lower numbers of profibrotic, IL-13-producing type II innate lymphoid cells and lower levels of IL-13 and fibrosis than wild-type mice. In tissue samples from patients with idiopathic pulmonary fibrosis (IPF), levels of IL-25, type II innate lymphoid cells and IL-13 were greater than those in tissue samples from patients without fibrotic lesions. Next steps could include testing IL-25 depletion in additional fibrosis models and developing IL-25-specific inhibitors.</p> <p>Amgen Inc., AstraZeneca plc and Kyowa Hakko Kirin Co. Ltd. have brodalumab, an IL-17 receptor (IL17R; IL17RA) antibody, in Phase III testing to treat psoriasis and psoriatic arthritis and Phase II trials to treat asthma.</p> <p>SciBX 7(5); doi:10.1038/scibx.2014.150 Published online Feb. 6, 2014</p>	Patent and licensing status undisclosed	<p>Hams, E. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Dec. 16, 2013; doi:10.1073/pnas.1315854111</p> <p>Contact: Padraic G. Fallon, Trinity College Dublin, Dublin, Ireland e-mail: pfallon@tcd.ie</p>
Respiratory distress syndrome (RDS)	Ras homolog family member A (RHOA)	<p>Cell culture and mouse studies suggest blocking nitration of RHOA could help treat acute lung injury (ALI) and RDS. In ALI and RDS, endothelial barrier function is compromised. In cultured endothelial cells, lipopolysaccharide (LPS) increased barrier disruption and RHOA activity compared with no treatment and resulted in the nitration of RHOA at Tyr34. In a mouse model of ALI, injection of a RHOA-derived peptide containing Tyr34 prior to injury blocked Rho nitration and decreased pathological changes compared with a control peptide injection. Next steps include developing additional peptides and determining whether they can reverse the disease course in additional mouse models of ALI.</p> <p>SciBX 7(5); doi:10.1038/scibx.2014.151 Published online Feb. 6, 2014</p>	Patent application filed; unavailable for licensing	<p>Rafikov, R. <i>et al. J. Biol. Chem.</i>; published online Jan. 7, 2014; doi:10.1074/jbc.M114.547596</p> <p>Contact: Stephen M. Black, Georgia Regents University, Augusta, Ga. e-mail: sblack@gru.edu</p>

This week in techniques

THE DISTILLERY brings you this week's most essential scientific findings in techniques, distilled by *SciBX* editors from a weekly review of more than 400 papers in 41 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable. **This week** in techniques includes findings about research tools, disease models and manufacturing processes that have the potential to enable or improve all stages of drug discovery and development.

Approach	Summary	Licensing status	Publication and contact information
Assays & screens			
Multiplexed detection of proteins in fine-needle aspirates	A method of profiling multiple proteins in fine-needle aspirates could help monitor tumor responses to therapy. Human cancer cells were incubated with a cocktail of 90 antibodies, each tagged with a different nonhuman DNA barcode. Photocleavage and detection of the barcodes provided a readout on the expression of each target protein. In fine-needle aspirates from primary tumors, the method identified treatment-associated changes in tumor protein profiles in four patients who received phosphoinositide 3-kinase (PI3K) inhibitors. Planned work includes using the method to monitor treatment responses in clinical trials of cancer therapies. The DNA barcode detection system used in this study is manufactured by NanoString Technologies Inc.	Patented by Massachusetts General Hospital; licensed to NanoString Technologies	Ullal, A.V. <i>et al. Sci. Transl. Med.</i> ; published online Jan. 15, 2014; doi:10.1126/scitranslmed.3007361 Contact: Ralph Weissleder, Massachusetts General Hospital, Boston, Mass. e-mail: rweissleder@mg.harvard.edu Contact: Cesar M. Castro, same affiliation as above e-mail: castro.cesar@mg.harvard.edu
SciBX 7(5); doi:10.1038/scibx.2014.152 Published online Feb. 6, 2014			
Disease models			
A transgenic, human <i>pregnane X receptor (PXR)</i> -expressing mouse model of type 2 diabetes	Transgenic mice expressing human <i>PXR</i> could be used to study the role of <i>PXR</i> in type 2 diabetes and screen for drugs to treat the indication. In mice fed a high-fat diet, transgenic expression of human <i>PXR</i> increased glucose intolerance, levels of circulating insulin and glucose and other symptoms of diabetes and decreased obesity compared with wild-type mouse <i>Pxr</i> expression. Future studies could include assessing <i>PXR</i> activity in tissues associated with human diabetes and using the transgenic model to screen for <i>PXR</i> inhibitors.	Patent and licensing status unavailable	Spruiell, K. <i>et al. J. Biol. Chem.</i> ; published online Dec. 20, 2013; doi:10.1074/jbc.M113.494575 Contact: Maxwell A. Gyamfi, North Carolina Central University, Durham, N.C. e-mail: mgyamfi@ncu.edu
SciBX 7(5); doi:10.1038/scibx.2014.153 Published online Feb. 6, 2014			
Drug delivery			
Nanoparticle-encapsulated delivery devices for sustained, near infrared (NIR) light-inducible release of drugs	Rat studies suggest an NIR light-activated device could enable controlled, repeated, sustained drug delivery. The device incorporates a drug reservoir encapsulated by an ethylcellulose matrix containing heat-sensitive gold nanoparticles. In saline, continuous-wave NIR light or heat stimulated the nanoparticles to create a porous membrane and release drug in a sustained manner. In diabetic rats, subcutaneous implantation of the device loaded with insulin followed by activation with periodic laser pulses decreased blood glucose levels coincident with NIR light trigger compared with what was seen in saline-loaded controls or with untriggered devices. Next steps include developing formulations that can be triggered by lower laser powers to improve safety.	Patent application filed; available for licensing	Timko, B.P. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Jan. 13, 2014; doi:10.1073/pnas.1322651111 Contact: Daniel S. Kohane, Boston Children's Hospital, Boston, Mass. e-mail: daniel.kohane@childrens.harvard.edu
SciBX 7(5); doi:10.1038/scibx.2014.154 Published online Feb. 6, 2014			
Drug platforms			
Correction of ring chromosomes in patient-derived induced pluripotent stem (iPS) cells	Reprogramming patient fibroblasts into iPS cells could help correct ring chromosome abnormalities for gene therapy applications. In fibroblasts from a patient with Miller-Dieker syndrome with a heterozygous ring chromosome 17, 4 of 6 iPS cell clones generated from the fibroblasts lost the ring chromosome and gained a second, identical copy of the other chromosome 17 because of a compensatory uniparental disomy mechanism. Clones that retained the ring chromosome were not viable through multiple passages. The findings were replicated in cells from two additional patients with chromosome 13 rings. Next steps include testing whether the strategy can be applied to other types of chromosomal abnormalities.	Unpatented; unavailable for licensing	Bershteyn, M. <i>et al. Nature</i> ; published online Jan. 12, 2014; doi:10.1038/nature12923 Contact: Anthony Wynshaw-Boris, Case Western Reserve University, Cleveland, Ohio e-mail: ajw168@case.edu Contact: Shinya Yamanaka, Gladstone Institute of Cardiovascular Disease, San Francisco, Calif. e-mail: syamanaka@gladstone.ucsf.edu
SciBX 7(5); doi:10.1038/scibx.2014.155 Published online Feb. 6, 2014			

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Dendrimer-formulated short activating RNAs (saRNAs) to enhance CCAAT enhancer binding protein- α (CEBPA)-dependent gene expression in liver cancer	Cell culture and rat studies suggest dendrimer-formulated saRNAs that bind target gene promoters could help treat cancer. CEPBA-saRNA was conjugated to a triethanolamine core poly(amidoamine) dendrimer for <i>in vivo</i> delivery. In a liver cancer cell line, CEPBA-saRNA increased secretion of albumin and decreased cell proliferation compared with a scrambled saRNA while maintaining hepatocyte function. In a cirrhotic rat model with associated liver cancer, a CEPBA-saRNA dendrimer conjugate increased levels of serum albumin and decreased tumor burden compared with saline or scrambled saRNA-dendrimer, suggesting improved liver function and antitumor activity. Next steps include developing an saRNA-nanoparticle candidate for clinical trials (<i>see RNA is for activation, page 8</i>).	Patents filed; available for licensing from MiNA Therapeutics Ltd. Contact: Robert Habib, MiNA Therapeutics Ltd., London, U.K. e-mail: robert@minatx.com	Reebye, V. <i>et al. Hepatology</i> ; published online Dec. 9, 2013; doi:10.1002/hep.26669 Contact: Nagy A. Habib, Imperial College London, London, U.K. e-mail: nagy.habib@imperial.ac.uk Contact: Vikash Reebye, same affiliation as above e-mail: v.reebye@imperial.ac.uk
Imaging			
2-Deoxy-2- ^{18}F fluoro-D-mannose (^{18}F FDM) to image high-risk atherosclerotic plaques with PET	Cell culture and rabbit experiments suggest ^{18}F FDM can be used to image high-risk atherosclerotic plaques. Macrophages are common in high-risk lesions and express the macrophage mannose receptor (MR). In cultured human macrophages, ^{18}F FDM uptake was higher than ^{18}F fluoro-D-glucose (^{18}F FDG) uptake. In a rabbit model of atherosclerosis, ^{18}F FDM labeled atherosclerotic lesions comparably in both <i>in vivo</i> and <i>ex vivo</i> imaging analyses. In the model, ^{18}F FDM uptake correlated with macrophage infiltration and was comparable to ^{18}F FDG uptake. Next steps include establishing methods for clinical-grade labeling of ^{18}F FDM and evaluating the reagent in human atherosclerotic plaques and carotid arteries.	Unpatented; licensing status not applicable	Tahara, N. <i>et al. Nat. Med.</i> ; published online Jan. 12, 2014; doi:10.1038/nm.3437 Contact: Jagat Narula, Icahn School of Medicine at Mount Sinai, New York, N.Y. e-mail: narula@mountsinai.org
<i>In vivo</i> imaging of CD8 $^{+}$ T cells using radiolabeled antibody fragments	Mouse studies suggest radiolabeled antibody fragments targeting CD8 could help monitor leukemia disease progression. Two antibody fragments based on mouse Cd8-depleting antibodies were designed that maintained antigen binding but did not deplete T cells. In normal mice, immuno-PET imaging detected accumulation of radiolabeled antibody fragments in the spleen and lymph nodes; the accumulation was decreased in Cd8-depleted, Cd8-blocked or immunodeficient mice. Next steps include designing an anti-human CD8 antibody fragment for clinical imaging.	Patent application filed; licensing negotiations ongoing; unavailable for licensing	Tavaré, R. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Jan. 3, 2014; doi:10.1073/pnas.1316922111 Contact: Anna M. Wu, University of California, Los Angeles, Calif. e-mail: awu@mednet.ucla.edu

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