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How to formulate and deliver therapeutic anti-microRNAs has been a big challenge in the burgeoning anti-miRNA space. Now, a San Diego team has proof of concept for a surprisingly simple way to deliver these molecules into the bloodstream using engineered B cells.

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By Tracey Baas, Senior Editor

National University of Singapore and **St. Jude Children's Research Hospital** team has engineered a T cell therapeutic that can engage in antibody-dependent cell cytotoxicity and enhance the effects of therapeutic antibodies regardless of the target tumor antigen.¹ The researchers showed proof of concept for boosting the efficacy of multiple marketed cancer antibodies.

They now plan to test T cell and antibody combinations in clinical trials in which antibody monotherapy did not generate remissions.

To create the T cell therapy, the team first designed a chimeric receptor that binds to the Fc portion of human antibodies and also delivers T cell activation signals.

The concept is similar to a chimeric antigen receptor (CAR) T cell, but rather than targeting a cancer-associated antigen, the NUS–St. Jude product targets the Fc portion of a human antibody that binds to a cancer-associated antigen.

Thus, with CAR-based T cells, the variability is in the T cell therapeutic. In contrast, the variability for the new chimeric receptor T cells is in the antibody therapeutic. As a result, only one type of engineered T cell is needed to treat multiple types of cancer, which simplifies manufacturing logistics.

The binding portion of the chimeric receptor was modeled after Fc γ -receptor III (CD16; FCGR3), which is found on NK cells and induces antibody-dependent cellular cytotoxicity (ADCC) when it engages with antibodies. The team reasoned that T cells expressing CD16 would engage in ADCC toward the cancer cells and selected a variant of CD16 with high affinity for antibodies.

Indeed, previous findings by a team from France showed that T cells engineered to express a CD16 and FCER1G (Fc fragment of IgE high affinity I receptor for γ -polypeptide) fusion protein induced ADCC against a cultured human B lymphoblast cell line in the presence of an anti-CD20 mAb.²

The signaling portion of the NUS–St. Jude chimeric receptor was made up of CD3 ζ and tumor necrosis factor receptor superfamily member 9 (TNFRSF9; 4-1BB; CD137), which induce T cell activation, proliferation and antitumor activity in T cells expressing antigen-specific CARs.³

The resulting product was T cells that express a universal chimeric receptor that targets the constant region of the Fc portion of antibodies.

The team first used a retroviral vector to express the CD16-BB- ζ construct—shorthand for the part CD16, part 4-1BB, part CD3 ζ construct—in human peripheral blood T lymphocytes and tested the cells' ability to enhance the efficacy of Rituxan rituximab against B cell malignancies.

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

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Rituxan/MabThera, an antibody against CD20 from **Biogen Idec Inc.** and the **Genentech Inc.** unit of **Roche**, is marketed for a variety of autoimmune diseases and hematological cancers.

In a cultured human B lymphoblast cell line, Rituxan plus CD16-BB- ζ -expressing T cells led to T cell activation, exocytosis of lytic granules and T cell proliferation, and the combination killed more than 50% of the target cells within 4 hours.

Importantly, the engineered T cells did not do anything in the absence of either the antibody or the antigen.

The next step was showing that the CD16-BB- ζ -expressing T cells could target more than one tumor antigen. Thus, the team tested the T cell and antibody combination in human breast and cancer cell lines expressing HER2 (EGFR2; ErbB2; neu) or human neuroblastoma and osteosarcoma cell lines expressing GD2, which is a target expressed on tumors of neuroectodermal origin.

Indeed, CD16-BB- ζ -expressing T cells plus Herceptin trastuzumab, which targets HER2, led to ADCC and cancer cell death. Similarly, CD16-BB- ζ -expressing T cells plus hu14.18K322A, an antibody from St. Jude that targets GD2, led to ADCC and cancer cell death. Genentech markets Herceptin to treat breast and gastric cancers. St. Jude's antibody is in Phase I

testing to treat neuroblastoma, melanoma and osteosarcoma.

T cells expressing CD16-BB- ζ induced significantly higher levels of ADCC than T cells expressing CD16 alone or those expressing the CD16-FCER1G fusion protein ($p < 0.0001$), as well as much more vigorous T cell expansion.

The strong *in vitro* results held up in mouse studies. In immunodeficient animals with human B lymphoblast cells, Rituxan followed by the engineered T cells resulted in remission in all 5 treated mice, whereas none of the 12 mice receiving engineered T cells or Rituxan alone survived.

Similar results were seen in immunodeficient mice with human neuroblastoma cells that received hu14.18K322A followed by the engineered T cells.

Because dramatic clinical results have been achieved with CAR-based cell therapies, the team set out to compare the cancer cell-killing potential of their universal CD16-BB- ζ T cells with that of CART cells that target CD19 and contain the same T cell signaling molecules.

Again, they tested the strategies in the cultured human B lymphoblast cell line, which expresses high levels of CD19 in addition to CD20.

In the cultured human B lymphoblast cell line, Rituxan plus CD16-BB- ζ -expressing T cells showed significantly greater cytotoxicity than Rituxan plus CD19-BB- ζ -expressing T cells ($p < 0.001$).

Results were published in *Cancer Research*.

“When you look at how many parties are trying to devise modular antibody-drug conjugate approaches to increase antibody effectiveness, it’s quickly clear how much of a value proposition this type of adaptable approach is. This approach leverages the ultimate effector mechanism: cytotoxic T cells.”

—*Michael Gladstone, Atlas Venture*

Moving to man

The team plans to test its approach in patients with B cell malignancies who previously received antibody therapy but are not in remission.

Instead of virus vector-mediated expression of the construct, the team plans to use electroporation to express its CD16-BB- ζ construct in the initial trials. “Electroporation adds an extra layer of safety to our system because the T cells will transiently express the construct,” said team leader Dario Campana, a professor of pediatrics at NUS. “As a second layer of safety, if any complications are seen, we can stop delivery of the immunotherapeutic antibody and ADCC will be abolished.”

The paper already showed that using electroporation is possible.

But other scientists suggested that the main safety concerns are on- or off-target effects of the antibodies that are selected for use with the engineered T cells.

“The new strategy’s specificity is only as good as the selectivity of the antibodies used. If these antibodies bind to antigens on nontumor tissues, it raises concerns about toxicity,” said Charles Sentman, a professor of microbiology and immunology at the **Geisel School of Medicine at Dartmouth**.

“The results indicate that universal Fc-CAR engineered T cells can significantly enhance ADCC potency and antitumor efficacy of antibody therapies. Potential safety concerns associated with such enhanced potency leading to increases in ‘on-target off-tumor’ toxicity directed toward normal tissue needs to be further evaluated,” added Madhusudan Peshwa, EVP of cellular therapies at **MaxCyte Inc.**

Carl June thinks that the biggest safety issue is whether the CD16-BB- ζ construct expressed on the T cells will also be triggered by circulating IgG that is not antigen bound *in vivo*.

IgG is the main type of antibody found in blood and extracellular fluid and can be induced by infections or cancer.

“The experiments were conducted in immunodeficient mice that had no circulating antibodies, so there is not yet sufficient preclinical toxicology studies available to know if this strategy will be able to move forward to human studies,” said June.

June is a professor in the Department of Pathology and Laboratory Medicine at the **Perelman School of Medicine at the University of Pennsylvania** and director of the translational research program at the **Abramson Family Cancer Research Institute at the University of Pennsylvania**.

“Binding of patient immune complexes to engineered CD16-BB- ζ -expressing T cells could potentially activate the T cells, resulting in killing of nearby normal cells and induction of inflammation which will not be directed against the targeted tumor,” added Sentman. “How significant this would be is unclear, but it is a potential risk that would need to be assessed.”

The problem, said Sentman, is that it is not obvious how to go about assessing that risk. “Human Fc receptors do not bind as well to many mouse IgGs, so testing in a mouse model may or may not be that informative,” he said.

“The experiments were conducted in immunodeficient mice that had no circulating antibodies, so there is not yet sufficient preclinical toxicology studies available to know if this strategy will be able to move forward to human studies.”

— **Carl June,**
Perelman School of Medicine at the
University of Pennsylvania

One approach, said Peshwa, would be to use immunodeficient mice co-infused with human IgGs and then test the antibody and T cell combination.

“T cells being triggered by circulating IgG is very unlikely,” said Campana. “Unbound antibody, such as circulating IgG, does not cause receptor cross-linking and hence should not trigger anything.”

Safety issues aside, Michael Gladstone, an associate in the life sciences group at **Atlas Venture**, said that the strategy “allows the unique opportunity to titrate the antibody dosing in a way that is virtually impossible to do with conventional CAR T cells that have a tumor-targeting protein locked in.”

The precision comes from the ability to dose specific amounts of antibodies. In contrast, it is difficult to predict how much expansion CAR T cells will undergo.

Gladstone said that the NUS–St. Jude technology is not entirely dissimilar from another type of immunotherapy—antibody-drug conjugates. Although virtually all antibody-drug conjugates employ some toxic payload, the academics essentially are using T cells as the payload, albeit without an actual chemical linker to

the antibody.

“When you look at how many parties are trying to devise modular antibody-drug conjugate approaches to increase antibody effectiveness, it’s quickly clear how much of a value proposition this type of adaptable approach is,” noted Gladstone. “This approach leverages the ultimate effector mechanism: cytotoxic T cells.”

NUS and St. Jude have jointly filed for patents covering the technology, and the IP is available for licensing.

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Abramson Family Cancer Research Institute at the University of Pennsylvania, Philadelphia, Pa.
Atlas Venture, Cambridge, Mass.
Biogen Idec Inc. (NASDAQ:BIIB), Weston, Mass.
Geisel School of Medicine at Dartmouth, Hanover, N.H.
Genentech Inc., South San Francisco, Calif.
MaxCyte Inc., Gaithersburg, Md.
National University of Singapore, Singapore
Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pa.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
St. Jude Children’s Research Hospital, Memphis, Tenn.

ClpPing persistence

By Chris Cain, Senior Writer

Northeastern University researchers have combined traditional antibiotics with compounds that activate bacterial clpP protease and cured mice with severe, highly drug-resistant *Staphylococcus* biofilm infections.¹ **Arietis Corp.** is developing analogs of the compounds to treat persistent bacterial infections.

Bacterial persistence occurs when a subpopulation of bacteria slows its growth rate and becomes insensitive to growth-inhibiting antibiotics. One place where this commonly occurs is in biofilms, which are surface-attached bacterial communities held together by extracellular polymeric matrices.

In particular, some *Staphylococcus aureus* infections are notorious for their persistence in the face of antibiotics, including those associated with endocarditis, osteomyelitis and implanted medical devices.

Kim Lewis, a professor and director of the Antimicrobial Discovery Center at Northeastern, told *SciBX* that a longtime lack of success in developing effective treatments for persistent infections led his team to attack the problem from a different angle.

“We knew from years of work on persisters that pathways for their formation are highly redundant and that trying to find a compound that inhibited persister formation was not going to be productive—we effectively learned what not to do,” he said.

“Persisters are dormant and in a metabolic state where established antibiotic targets are shut down, so antibiotics that inhibit essential proteins are not going to work,” he continued. “That left us with one possibility—to look for something that is going to activate and corrupt an essential function without requiring energy. This seemed like a tall order, but we found a compound that seemed to match this criterion.”

His team homed in on a class of molecules called acyldepsipeptides (ADEPs), which are natural products that kill bacteria by binding and activating the clpP protease, according to 2005 findings by **Bayer AG**.² ClpP is a key regulator of bacterial protein homeostasis that is conserved in most bacteria. Its activity is normally tightly regulated and requires an ATPase or another accessory protein to initiate proteolysis. ADEPs allow clpP to bypass these regulatory processes, resulting in uncontrolled proteolysis and ultimately cell death.

To test whether ADEPs could help target persistence, Lewis’ team incubated an ADEP analog called ADEP4 with *S. aureus* grown *in vitro* to stationary phase, in which the cells no longer divide and become metabolically inactive. Over a three-day treatment period, ADEP4 significantly reduced the number of viable bacteria, whereas the traditional antibiotics ciprofloxacin, vancomycin, linezolid and rifampicin had no effect.

Proteomic analyses confirmed that incubation with ADEP4 triggered extensive protein degradation in stationary phase bacteria.

When ADEP4 was combined with rifampicin, linezolid or ciprofloxacin, *S. aureus* was completely eliminated from the culture. This result was repeated when ADEP4 plus rifampicin was tested against a variety of *S. aureus* strains, including methicillin-resistant *S. aureus* (MRSA).

Finally, the group turned to a mouse model of severe *S. aureus* infection. In the model, mice are first treated with cyclophosphamide to suppress the immune system, and then a large dose of *S. aureus* is injected into the thigh and allowed to incubate for 24 hours before antibiotic treatment.

ADEP4 plus rifampicin completely eliminated infection in all five mice, whereas animals treated with rifampicin, vancomycin or a combination of the two had reduced numbers of bacteria but did not clear the infection.

Results were published in *Nature*.

Resistance questions

Jason Sello, an associate professor of chemistry at **Brown University**, noted that the results highlight a conceptually new approach to fighting persistent bacterial infections.

“The findings are indeed quite remarkable. The reported observations indicate that metabolically quiescent yet imminently lethal bacteria can be killed by activation of endogenous enzymes that degrade macromolecules,” he said.

Sello’s group has developed inhibitors of clpP that kill *Mycobacterium tuberculosis*³ and analogs of the ADEP activators of clpP that have enhanced pharmacological properties and are active against *S. aureus*, *Streptococcus pneumoniae* and Enterococci. ClpP is an essential gene in *M. tuberculosis*.

That is not the case in many bacterial species, including *S. aureus*, in which, unlike the essential protein complexes inhibited by traditional antibiotics, clpP is not essential for bacterial growth *in vitro*. This means it is

relatively common for strains to develop resistance to ADEPs *in vitro* by acquiring inactivating mutations in clpP.

Thus, an important next question for ADEPs and other clpP-targeting compounds is whether the development of resistance can be overcome by combination strategies.

Scott Gray-Owen, a professor of molecular genetics at the **University of Toronto**, is not convinced that the resistance often seen *in vitro* means it will develop *in vivo*. “Just because we can knock the gene out and the bacteria can grow *in vitro*, it does not mean that the bacteria will necessarily be infectious anymore. The ‘essential’ nature of genes is often defined by growth in extremely rich media in the lab and may not represent their role in the host,” he said.

Gray-Owen is collaborating with Walid Houry, a professor of biochemistry at the University of Toronto, to develop compounds that activate clpP, including molecules specific for particular bacterial strains.⁴

“Persisters are dormant and in a metabolic state where established antibiotic targets are shut down, so antibiotics that inhibit essential proteins are not going to work. That left us with one possibility—to look for something that is going to activate and corrupt an essential function without requiring energy. This seemed like a tall order, but we found a compound that seemed to match this criterion.”

**—Kim Lewis,
Northeastern University**

Lewis said that *clpP*-mutant *S. aureus* is highly attenuated, and in the *Nature* paper his team showed that the mutants were more sensitive to traditional antibiotics than wild-type cells. He also noted that if an entire

bacterial population, including persisters, is rapidly wiped out by combination treatment, there would be less time for resistance to emerge.

Houry agreed. "If this approach is working on the dormant bacteria that are residing in the background, if you kill them off in the first pass before they begin to grow, then resistance may not emerge," he said.

Another question is whether activating *clpP* could help treat persistent Gram-negative infections, such as *Pseudomonas aeruginosa*. Lewis said that ADEPs do not effectively penetrate the membrane of Gram-negative bacteria, so distinct compounds would need to be developed to test that hypothesis.

Prabhavathi Fernandes, founder, president and CEO of **Cempra Inc.**, was enthusiastic about the findings but wanted to see more characterization of ADEP4, including resistance frequency and pharmacokinetic data, and particularly more data on the safety profile of the ADEPs after longer-term dosing studies. "The results are thought provoking and certainly remarkable. There is an urgent need for antibiotics that can overcome biofilm infections," she said. "It's early but beautiful work."

She added that pharmacokinetics would be particularly important if the drug were to be used in combination with other antibiotics. "Both drugs have to be in the right place at the right time to prevent the resistance; if one goes away you get resistance. In a sense each drug is protecting the other," she said.

Fernandes and Sello both said that they would like to see the compound tested in combination with other commonly used classes of antibiotics, including β -lactams with β -lactamase (LACTB) inhibitors.

Cempra's solithromycin, a macrolide antibiotic, is in Phase III testing for community-acquired bacterial pneumonia (CABP). The company's Taksta (fusidic acid) has completed Phase II trials for acute bacterial skin and skin structure infections (ABSSSIs) and is in Phase II testing to treat prosthetic joint infections, in which it has received orphan designation.

"The results are thought provoking and certainly remarkable. There is an urgent need for antibiotics that can overcome biofilm infection."

—Prabhavathi Fernandes,
Cempra Inc.

Arietis, an antibiotic company founded by Lewis in 2008, is generating pharmacology and safety data for ADEPs and has exclusively licensed patents covering analogs and their combination with traditional antibiotics from Northeastern University.

"We are performing target validation studies on *clpP* and actively looking for other antimicrobials that function by activating a cellular process," said COO Michael LaFleur. "We are also performing preclinical pharmacokinetic, pharmacodynamic and safety assessments on ADEPs and are working with a medicinal chemistry group on analogs of ADEP with improved pharmacological properties."

LaFleur said that the company has received more than \$6 million in Small Business Innovation Research (SBIR) grants since 2008 to fund its research.

Arietis plans to identify compounds that can kill bacteria by activating or corrupting the function of additional targets, and Lewis' lab is continuing to study mechanisms of persistence.

Gray-Owen said that other bacterial proteases could be attractive candidates to activate to kill persistent bacteria. However, he said that proof of concept for activating *clpP* was only possible because of years of basic work in understanding how ADEPs kill bacteria.

"This absolutely rationalizes basic biology research; no one would have thought to rationally design a compound with this mechanism of action. It would be hard to predict that dysregulating this complex would kill bacteria. That is why it had to emerge from basic biological insights into the mechanism of ADEP killing," he said.

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COMPANIES AND INSTITUTIONS MENTIONED

Arietis Corp., Boston, Mass.
Bayer AG (Xetra:BAYN), Leverkusen, Germany
Brown University, Providence, R.I.
Cempra Inc. (NASDAQ:CEMP), Chapel Hill, N.C.
Northeastern University, Boston, Mass.
University of Toronto, Toronto, Ontario, Canada

Plan B for anti-miRNA

By Lev Osherovich, Senior Writer

Formulating and delivering therapeutic anti-microRNAs has been a big challenge in the burgeoning anti-miRNA space. Now, a **University of California, San Diego** team has proof of concept for a surprisingly simple way to deliver these molecules into the bloodstream using engineered B cells.¹

The UCSD group, led by Professor of Medicine Maurizio Zanetti, used murine B cells transfected with an RNA-encoding construct to knock down expression of an endogenous miRNA in T cells, which work closely with B cells during immune activity (see **Figure 1**, “Anti-miRNA delivery by B cells”).

The new delivery technique exploits exosomes—small, lipid-bound vesicles that are naturally secreted by a variety of cells—to transfer the anti-miRNA from the engineered B cells to the target cells.

“The novelty is that you can use a cell that is particularly abundant in the blood to deliver a customized immunogenomic treatment,” said Zanetti. “You can imagine exploiting this property to attack different disease processes, be it cancer, immune response or inflammation.”

Determining whether the technique can target RNA in other cells besides T cells and whether the magnitude of the knockdown is enough for a therapeutic effect will require further preclinical work.

Exosome relay

Zanetti’s team started by harvesting B cells from mice and transfecting them with a plasmid encoding a 22-base-pair sequence that was complementary and thus antagonistic to miR-150. miR-150 influences both B and T cell differentiation.

In cell culture, transfected B cells produced large amounts of anti-miR-150 RNA and released the transcript into the medium. The RNA was primarily encapsulated in exosomes secreted by the B cells.

Next, the group simulated conditions that activate T cells *in vitro* by coculturing dendritic cells and T cells in media previously used to grow the anti-miR-150-transfected B cells.

In the presence of a peptide antigen, T cells became activated and absorbed B cell–derived exosomes. As a result, anti-miR-150 penetrated into the T cells and knocked down expression of *miR-150* by about 70%, whereas mock-treated control cells showed no knockdown.

Similar results occurred *in vivo*. T cells from the spleens of mice that

received anti-miR-150-transfected B cells had about 60% lower miR-150 than control cells.

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

Special delivery

Zanetti’s findings demonstrate the feasibility of using B cells to manufacture bioavailable anti-miRNAs. However, many questions remain about whether the technique would be effective beyond the specific scenario of T cell activation.

Neil Gibson, CSO of miRNA company **Regulus Therapeutics Inc.**, said that the principal novelty of Zanetti’s study is that transfected B cells can produce functional anti-miRNAs.

Most techniques in clinical development for knocking down RNA rely on synthetic lipid nanoparticles or chemical conjugates to shield the therapeutic RNA molecules from degradation or excretion. There are no disclosed RNA-targeted therapeutic candidates in the clinic that specifically target B or T cells.

“We had in general thought you couldn’t deliver to B cells, though

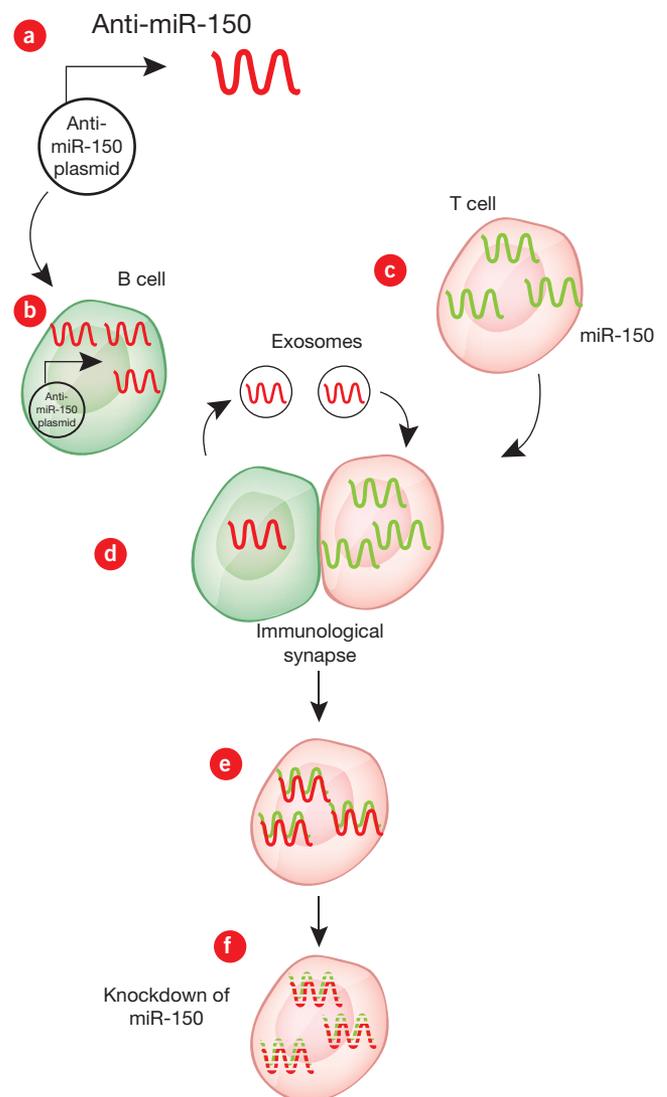


Figure 1. Anti-miRNA delivery by B cells. Almanza *et al.* have engineered B cells to deliver anti-microRNA to other immune cells. The findings suggest a new avenue for delivering therapeutic miRNA.

A plasmid (a) engineered to encode a short sequence complementary to microRNA-150 (anti-miR-150) is transfected in murine B cells (b). In cell culture and *in vivo*, engineered B cells interact with T cells, which naturally express miR-150 (c). When B cells and T cells interact to form an immunological synapse (d), anti-miR-150 is secreted into membrane-bound extracellular vesicles (exosomes), which are absorbed by the T cells (e). This leads to degradation and knockdown of endogenous miR-150 (f).

nanoparticle and conjugate strategies have been tried,” said Gibson. “But this is taking another step where you use the B cells almost as manufacturing machines for nucleic acid therapeutics.”

Gibson said that the study suggests exosomes “are the critical mechanism for transfer of anti-miRNAs. This raises the possibility that if you have a cell with a high capacity for generating exosomes, this could be a way to deliver anti-miRNAs to other cell types that are in close proximity.”

Other cell types could include a variety of T cells, innate immune cells and possibly cancer cells.

Regulus’ most advanced candidate is RG-101, an anti-miRNA therapeutic that will enter Phase I testing for HCV in 2014.

Zanetti thinks that engineered B cells could be used as autologous cell therapy for a range of autoimmune, inflammatory and cancer indications.

“B cells have the capacity to internalize plasmid or bacterial DNA and are relatively abundant—about 15% of cells in blood—so you don’t have to scale them up,” said Zanetti.

It is unclear how long B cell-derived exosomes and their anti-miRNA cargo will persist *in vivo* and whether other cell types besides T cells can take them up. Details on the *in vivo* properties of anti-miR-150 were not reported in the *PNAS* paper.

“They show that the B cells are producing anti-miRNA, but will they get enough secretion to do something useful?” asked Matthew Scholz, founder and CEO of **Immusoft Corp.**

Immusoft is developing a platform for *in vivo*, B cell-based manufacturing of antiviral mAbs and enzyme-replacement therapies.

“B cells can persist and produce their payload for a few weeks,” said Scholz, but whether the amount of anti-miRNA produced by Zanetti’s transfected B cells is an effective dose in actual disease needs to be determined.

Scholz also wanted to see side-by-side comparisons of anti-miRNAs from engineered B cells with current synthetic lipid formulations “to see if the exosomes secreted naturally are equivalent to some of the synthetic liposomes.”

He noted that pharmacodynamic data about Zanetti’s anti-miRNAs could make or break the case for using B cell-derived RNA over conventional *in vitro* formulations. If the pharmacodynamics of the two

types of anti-miRNA are comparable, Scholz suspects that “the B cells may not even be necessary.”

Gibson said that Zanetti’s next step should be to test the engineered B cells in a disease model. He suggested inflammatory or autoimmune indications such as rheumatoid arthritis (RA), in which dysregulated T cells play a critical role.

“There are clearly opportunities in the immune modulation space to see whether you can sensitize the immune system” with anti-miRNAs, said Gibson.

Zanetti agreed, adding that B cell-derived anti-miRNAs could also be useful in cancer immunotherapy provided that the B cells can be locally delivered to the tumor site.

“The regulation of the immune system such as proposed in the paper is one therapeutic possibility,” he said. “The other is to target cancer tissues and solid tumors, but you would have to improve the targeting of B cells to tumors.”

The **University of California** has filed patents on the technique, and the IP is available for licensing.

“We had in general thought you couldn’t deliver to B cells, though nanoparticle and conjugate strategies have been tried. But this is taking another step where you use the B cells almost as manufacturing machines for nucleic acid therapeutics.”

—Neil Gibson, Regulus Therapeutics Inc.

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Contact: Maurizio Zanetti, University of California, San Diego, La Jolla, Calif.
e-mail: mzanetti@ucsd.edu

COMPANIES AND INSTITUTIONS MENTIONED

Immusoft Corp., Seattle, Wash.
Regulus Therapeutics Inc. (NASDAQ:RGLS), San Diego, Calif.
University of California, Oakland, Calif.
University of California, San Diego, La Jolla, Calif.

Family seeds

By Amy Donner, Senior Editor

It has been long assumed that the specificity of microRNA inhibitors is determined by length—dogma held that anti-miRNAs should be more than 16 nucleotides and approximate the length of the actual miRNA targets. Now, an international team has *in vivo* proof of concept that using anti-miRNAs of only eight nucleotides in length does not compromise selectivity and provides a simple tool for blocking multiple miRNAs at once.¹

The researchers are planning a Phase I trial following additional toxicology studies in animals.

miRNAs are small noncoding RNAs that regulate gene expression by binding to specific mRNAs via complementary base pairing. Typically, a miRNA regulates one or several different mRNAs. In addition, an mRNA can be targeted by several miRNAs. This regulatory redundancy makes it challenging to intervene therapeutically.

Affinity and specificity of binding between miRNAs and mRNAs is driven in large part by complementary base pairing interactions. Thus, most companies developing anti-miRNAs have opted to focus on molecules that approximate the length of their miRNA targets to maximize such interactions.

Santaris Pharma A/S has the only disclosed anti-miRNA in the clinic. The company's miravirsin, an anti-miRNA targeting miR-122, is in Phase II trials to treat HCV.

Several companies have preclinical anti-miRNA programs, including **Regulus Therapeutics Inc.**'s anti-miRNA that simultaneously targets miR-33a and miR-33b, which control genes involved in regulating cholesterol.²

Regulus is developing its miR-33-targeting anti-miRNA preclinical program in collaboration with **AstraZeneca plc**.

Recently, researchers at Santaris, **Cold Spring Harbor Laboratory** and **Aalborg University** proposed an alternative strategy for simultaneously inhibiting multiple miRNAs by using 8mer anti-miRNAs against a region of 6–8 nucleotides called the seed region that is responsible for mRNA target selection.³

Seed regions tend to be conserved among redundant miRNAs. The approach was shown to work *in vitro* and in mice.⁴

One major advantage of developing shorter, seed-targeting anti-miRNAs is that they can be rationally designed. In contrast, their longer counterparts need to be developed empirically.

Now, a group led by Anders Näär has tested the 'shorter is better' strategy in a nonhuman primate model and shown that it has therapeutic potential. The team used an 8mer anti-miRNA oligonucleotide that targets the seed region of miR-33a and miR-33b.

Näär is a professor of cell biology at **Harvard Medical School** and the **Massachusetts General Hospital Cancer Center**.

First, Näär's team screened an anti-miRNA library and isolated a locked nucleic acid (LNA)-modified, 8mer anti-miRNA that inhibited miR-33a and miR-33b activity in binding and gene expression assays.

Next, the group tested the activity and specificity of the anti-miR-33a/b

in cells and mice. The 8mer de-repressed miR-33a and miR-33b at the RNA and protein level in a human liver cell line, and it increased high-density lipoprotein (HDL) levels in mice compared with saline.

In obese African green monkeys fed a high-fat diet, subcutaneous injection of anti-miR-33a/b increased HDL up to 39% compared with vehicle. Liver biopsies showed that anti-miR-33a/b de-repressed several miR-33a and miR-33b targets.

Anti-miR-33a/b was well tolerated for more than 108 days and had a plasma half-life of 17.5 days, which is comparable to half-lives reported for longer anti-miRNAs. There were no injection site reactions or adverse effects.

These results were similar to nonhuman primate data reported by Regulus showing the effects of longer anti-miRNAs targeting miR-33a and miR-33b. Regulus researchers also saw increased hepatic expression of miR-33a and miR-33b targets and higher plasma levels of HDL cholesterol compared with using mismatch control anti-miRNA.⁴

The new study was published in *Science Translational Medicine*. The team included researchers from Santaris, **Aalborg University Hospital**, **Duke University Medical Center**, **RxGen Inc.** and **Weill Cornell Medical College in Qatar**.

Santaris was involved in every stage of the study but did not respond to requests for comment. RxGen carried out the nonhuman primate studies.

“The nonhuman primate study is an important step toward development of therapeutics inhibiting entire miRNA families sharing the same seed sequence.”

—**Sakari Kauppinen,**
Aalborg University Hospital

“The nonhuman primate study is an important step toward development of therapeutics inhibiting entire miRNA families sharing the same seed sequence,” said study coleader Sakari Kauppinen, a professor of hematology at Aalborg University Hospital.

Targeting the seed

Näär's group now plans to run toxicology studies in rodents and nonhuman primates

prior to a Phase I safety trial. The next step would be testing anti-miR-33a/b therapy in patients with familial hypercholesterolemia.

According to Näär, about 47% of all conserved miRNAs are members of families sharing the same seed sequence, suggesting that this approach could be useful in other diseases associated with miRNA families. The ability to target multiple miRNAs safely and efficaciously using a single 8mer therapeutic represents an important alternative approach for the field.

Neil Gibson, CSO of Regulus, said that papers about 8mers “establish proof of concept of therapeutic potential. The latest study provides additional compelling evidence that the miRNA-targeting approach is safe and well tolerated.”

Gibson added that targeting the seed is not the only strategy for hitting families of miRNAs.

“All miRNAs in a family do not necessarily interact with the same mRNAs. The way you target the family may be strongly influenced by the mRNAs you are trying to modulate. With longer anti-miRNAs you can differentially regulate members of the same family, taking advantage of both seed and nonseed sequences. Integration of chemical modifications can also help you control selectivity of anti-miRNAs for miRNAs,” he said.

Massachusetts General Hospital has filed for patents in the U.S., Europe and Japan covering the therapeutic targeting of miR-33a and

miR-33b to treat cholesterol-related disorders and for regulating lipid metabolism. The patent applications include anti-miRNAs from 8mer up to full complementarity with miR-33a and miR-33b.

Regulus' patent portfolio includes patents and patent applications covering the sequences and complementary sequences of anti-miR-33a/b, composition of matter covering various chemically modified anti-miRNAs and method of use for targeting miR-33a and miR-33b to treat cardiovascular disease.

Näär acknowledged that "the miR-33 IP landscape is complex. I think MGH is on solid ground, but anyone interested in licensing our miR-33 IP will obviously do their own due diligence."

Donner, A. *SciBX* 6(47); doi:10.1038/scibx.2013.1340
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Contact: Anders M. Näär, Massachusetts General Hospital Cancer Center, Charlestown, Mass.
e-mail: naar@helix.mgh.harvard.edu

Contact: Sakari Kauppinen, Santaris Pharma A/S, Hørsholm, Denmark
e-mail: sk@bio.aau.dk

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COMPANIES AND INSTITUTIONS MENTIONED

Aalborg University, Ballerup, Denmark
Aalborg University Hospital, Copenhagen, Denmark
AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
Duke University Medical Center, Durham, N.C.
Harvard Medical School, Boston, Mass.
Massachusetts General Hospital, Charlestown, Mass.
Massachusetts General Hospital Cancer Center, Charlestown, Mass.
Regulus Therapeutics Inc. (NASDAQ:RGLS), San Diego, Calif.
RxGen Inc., Hamden, Conn.
Santaris Pharma A/S, Hørsholm, Denmark
Weill Cornell Medical College in Qatar, Doha, Qatar



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This week in therapeutics

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

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

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer				
Cancer	Enhancer of zeste homolog 2 (EZH2)	<p>Cell culture studies identified a small molecule inhibitor of EZH2 that could help treat EZH2-dependent cancers. Chemical screening and lead optimization identified a competitive small molecule inhibitor that selectively decreased trimethylation of histone H3K27 by wild-type or mutant EZH2. In Pfeiffer lymphoma cells carrying mutant EZH2, the lead compound dose-dependently inhibited growth and H3K27 trimethylation. Next steps include further optimizing lead compounds and testing them in lymphoma models.</p> <p>Epizyme Inc. has the selective EZH2 inhibitor E7438 in Phase I/II testing to treat lymphomas.</p> <p>Constellation Pharmaceuticals Inc. has EZH2 inhibitors in discovery to treat cancer.</p> <p>GlaxoSmithKline plc and Novartis AG also have EZH2 discovery programs.</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1341 Published online Dec. 12, 2013</p>	Patent and licensing status undisclosed	<p>Garapaty-Rao, S. <i>et al. Chem. Biol.</i>; published online Oct. 31, 2013; doi:10.1016/j.chembiol.2013.09.013</p> <p>Contact: Patrick Trojer, Constellation Pharmaceuticals Inc., Cambridge, Mass. e-mail: patrick.trojer@constellationpharma.com</p>
Cancer	Histone deacetylase (HDAC); interferon- γ (IFN γ)	<p>Mouse studies suggest combining HDAC inhibitors with IFN-γ stimulation may enhance antitumor efficacy. In immune-deficient mouse models of colon adenocarcinoma and lymphoma, an HDAC inhibitor had less potent antitumor activity than that seen in immunocompetent mice. In the lymphoma models, dual treatment with HDAC inhibitors and the Ifn-γ-inducing agent α-GalCer increased survival compared with treatment using either agent alone. Next steps could include testing combined HDAC inhibition and immune stimulation in clinical trials.</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1342 Published online Dec. 12, 2013</p>	Patent and licensing status unavailable	<p>West, A.C. <i>et al. Cancer Res.</i>; published online Oct. 24, 2013; doi:10.1158/0008-5472.CAN-13-0890</p> <p>Contact: Ricky W. Johnstone, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia e-mail: ricky.johnstone@petermac.org</p>
Colorectal cancer (CRC)	B cell lymphoma 2 (BCL-2; BCL2); Bcl-x _L ; mammalian target of rapamycin complex 1 (mTORC1); mTORC2	<p>Cell culture and mouse studies suggest antagonizing mTORC1 or mTORC2 in combination with BCL-2 and Bcl-x_L could be useful for treating K-Ras (KRAS)- or BRAF-mutant CRC. In cell culture and two mouse models of CRC, a combination of the BCL-2 and Bcl-x_L inhibitor navitoclax (ABT-263) and a pan-mTOR (FRAP; RAFT1) inhibitor decreased growth of KRAS- and BRAF-mutant tumors but not that of tumors with wild-type KRAS or BRAF. Next steps could include preclinical optimization of a combination therapy with agents in clinical development. AbbVie Inc.'s ABT-263 is in Phase I/II testing for advanced small cell lung cancer (SCLC).</p> <p>At least a dozen BCL-2 and Bcl-x_L inhibitors are in Phase II or earlier development for various cancers.</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1343 Published online Dec. 12, 2013</p>	Patent and licensing status undisclosed	<p>Faber, A.C. <i>et al. Cancer Discov.</i>; published online Oct. 25, 2013; doi:10.1158/2159-8290.CD-13-0315</p> <p>Contact: Jeffrey A. Engelman, Massachusetts General Hospital Cancer Center, Boston, Mass. e-mail: jengelman@partners.org</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Mantle cell lymphoma (MCL)	Unknown	<p>Mouse studies suggest Revlimid lenalidomide helps treat MCL by inhibiting lymphangiogenesis. In mouse xenograft models of MCL, Revlimid inhibited tumor growth and decreased lymphangiogenesis, the number of tumor-associated macrophages and cancer dissemination to auxiliary lymph nodes compared with vehicle. Next steps could include validating the mechanism in additional animal models and exploring other methods of disrupting lymphangiogenesis to treat MCL.</p> <p>Celgene Corp.'s Revlimid is approved to treat MCL, multiple myeloma (MM) and myelodysplastic syndrome (MDS).</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1344 Published online Dec. 12, 2013</p>	Patent and licensing status unavailable	<p>Song, K. <i>et al. Cancer Res.</i>; published online Oct. 24, 2013; doi:10.1158/0008-5472.CAN-13-0750 Contact: Lijun Xia, Oklahoma Medical Research Foundation, Oklahoma City, Okla. e-mail: lijun-xia@omrf.org</p>
Non-small cell lung cancer (NSCLC)	Lysine-specific demethylase 2A (KDM2A; JHDM1A)	<p>Patient sample, cell culture and mouse studies suggest decreasing levels of KDM2A could help treat NSCLC. In patient NSCLC tumor samples, KDM2A mRNA and protein levels were higher than those in normal lung tissue samples and correlated with poor survival. In NSCLC cell lines expressing high levels of KDM2A, siRNA against <i>KDM2A</i> decreased cell proliferation and invasiveness compared with control siRNA. In mice injected with NSCLC cells with high KDM2A levels, pretreatment with siRNAs against <i>KDM2A</i> led to no lung tumors, whereas control siRNA pretreatment led to multiple lung tumors. Next steps include using lung-specific knockout or transgenic mouse models to provide genetic evidence that KDM2A promotes NSCLC.</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1345 Published online Dec. 12, 2013</p>	Patent application filed; available for licensing	<p>Wagner, K.W. <i>et al. J. Clin. Invest.</i>; published online Nov. 8, 2013; doi:10.1172/JCI68642 Contact: Min Gyu Lee, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: mglee@mdanderson.org</p>
Prostate cancer	IL-6; signal transducer and activator of transcription 3 (STAT3)	<p><i>In vitro</i> and mouse studies suggest inhibiting IL-6 or STAT3 could help treat castration-resistant prostate cancer. In a mouse model of prostate cancer, repeated treatment with the generic androgen receptor antagonist flutamide increased STAT3 activation and expression compared with vehicle treatment and led to drug resistance. In the mice, shRNA against STAT3 or treatment with a soluble IL-6 receptor (CD126) delayed tumor growth, whereas shRNA control or vehicle treatment did not. Soluble IL-6 receptor plus the androgen receptor antagonist Casodex bicalutamide decreased tumor growth compared with either treatment alone. Next steps could include testing the strategy in additional animal models.</p> <p>At least 11 companies have IL-6 antibodies or inhibitors in Phase III or earlier testing.</p> <p>AstraZeneca plc and Isis Pharmaceuticals Inc. have AZD9150, an antisense inhibitor of STAT3, in Phase II trials to treat cancer.</p> <p>At least five other companies have STAT3 inhibitors in Phase I testing or earlier to treat cancers.</p> <p>AstraZeneca markets Casodex to treat prostate cancer.</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1346 Published online Dec. 12, 2013</p>	Patent and licensing status unavailable	<p>Schroeder, A. <i>et al. Cancer Res.</i>; published online Oct. 31, 2013; doi:10.1158/0008-5472.CAN-13-0594 Contact: Anne Schroeder, Beckman Research Institute at City of Hope, Duarte, Calif. e-mail: aschroder@coh.org Contact: Richard Jove, same affiliation as above e-mail: r.jove@coh.org Contact: Gehard Mueller-Newen, RWTH Aachen University, Aachen, Germany e-mail: mueller-newen@rwth-aachen.de</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Skin cancer	BRAF; c-jun N-terminal kinase (JNK)	<i>In vitro</i> and mouse studies suggest the BRAF inhibitor Zelboraf vemurafenib could induce cutaneous squamous cell carcinoma (cSCC) by inhibiting JNK signaling. Treatment of melanoma with BRAF inhibitors can increase the incidence of cSCC. In cSCC cells and keratinocytes, Zelboraf suppressed JNK activation and apoptosis by inhibiting kinases upstream of JNK including sterile α -motif and leucine zipper containing kinase AZK (ZAK). Next steps include using this information to design melanoma therapeutics that do not reduce JNK signaling. Daiichi Sankyo Co. Ltd., Chugai Pharmaceutical Co. Ltd. and Roche market Zelboraf to treat melanoma. GlaxoSmithKline plc markets the BRAF inhibitor Tafenlar dabrafenib to treat melanoma. At least four other companies have BRAF inhibitors in Phase III or earlier testing to treat cancers. SciBX 6(47); doi:10.1038/scibx.2013.1347 Published online Dec. 12, 2013	Patent status not applicable; unavailable for licensing	Vin, H. <i>et al. eLife</i> ; published online Nov. 5, 2013; doi:10.7554/eLife.00969 Contact: Kenneth Y. Tsai, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: kytsai@mdanderson.org
Cardiovascular disease				
Arterial thrombosis; thrombosis	Purinergic receptor P2Y G protein-coupled 1 (P2RY1; P2Y1)	<i>In vitro</i> and rat studies have identified P2Y1 antagonists that could help treat thrombosis. Chemical synthesis, SAR and <i>in vitro</i> testing of <i>ortho</i> -anilino diaryl urea analogs identified several inhibitors of P2Y1 that blocked platelet aggregation <i>ex vivo</i> in human plasma at low nanomolar IC ₅₀ values. In rat models of arterial thrombosis and venous thrombosis, one compound decreased thrombus formation and increased blood flow compared with vehicle. Future work could include optimizing the bioavailability and other pharmacokinetic parameters of the lead compound. SciBX 6(47); doi:10.1038/scibx.2013.1348 Published online Dec. 12, 2013	Patent and licensing status unavailable	Qiao, J.X. <i>et al. J. Med. Chem.</i> ; published online Oct. 28, 2013; doi:10.1021/jm4013906 Contact: Jennifer X. Qiao, Bristol-Myers Squibb Co., Pennington, N.J. e-mail: jennifer.qiao@bms.com
Cardiovascular disease	MicroRNA-33a (miR-33a); miR-33b	Mouse and primate studies suggest an 8mer anti-miRNA that targets both miR-33a and miR-33b could help treat cardiovascular disease. miR-33a and miR-33b inhibit the expression of genes involved in lipid metabolism and insulin signaling. In mice, an 8mer anti-miRNA targeting the seed regions of miR-33a and miR-33b (anti-miR-33a/b) increased high-density lipoprotein (HDL) cholesterol compared with a scrambled anti-miRNA. In nonhuman primate models of metabolic disease, anti-miR-33a/b increased HDL cholesterol up to 39% compared with vehicle or anti-miRNAs targeting either miR-33a or miR-33b. In primates treated for more than 100 days, there were no adverse events, injection site reactions, liver inflammation or fibrosis. Next steps include toxicology studies in rodents and nonhuman primates. Regulus Therapeutics Inc. and AstraZeneca plc have an anti-miRNA targeting miR-33 in preclinical testing to treat cardiovascular disease (see <i>Family seeds</i> , page 8). SciBX 6(47); doi:10.1038/scibx.2013.1349 Published online Dec. 12, 2013	Patent application filed covering therapeutic targeting of miR-33a and miR-33b for treatment of lipid-related disorders; available for licensing	Rottiers, V. <i>et al. Sci. Transl. Med.</i> ; published online Nov. 20, 2013; doi:10.1126/scitranslmed.3006840 Contact: Anders M. Näär, Massachusetts General Hospital Cancer Center, Charlestown, Mass. e-mail: naar@helix.mgh.harvard.edu Contact: Sakari Kauppinen, Santaris Pharma A/S, Hørsholm, Denmark e-mail: sk@bio.aau.dk

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Ischemia/reperfusion injury	Protein tyrosine phosphatase non-receptor type 5 (PTPN5; STEP); p38 mitogen-activated protein kinase (p38 MAPK; MAPK14)	Rat studies identified a degradation-resistant STEP peptide that could help treat ischemia/reperfusion injury. In rats, transient ischemia activated p38 MAPK and initially increased levels of the p38 inhibitor STEP compared with no ischemia, although STEP was eventually degraded. In the rat model, i.v. injection of a cell-permeable, degradation-resistant TAT-STEP-c-Myc (MYC) peptide decreased both p38 MAPK activation and brain damage compared with vehicle injection. Next steps include testing the effects of the peptide on long-term recovery and in stroke models with significant comorbidities. SciBX 6(47); doi:10.1038/scibx.2013.1350 Published online Dec. 12, 2013	Patent application filed; licensed by Tyrosine Pharma	Deb, I. <i>et al. J. Neurosci.</i> ; published online Nov. 6, 2013; doi:10.1523/JNEUROSCI.2346-12.2013 Contact: Surojit Paul, University of New Mexico, Albuquerque, N.M. e-mail: spaul@salud.unm.edu
Endocrine/metabolic disease				
Diabetes	Nicotinic acid adenine dinucleotide phosphate (NAADP)	Cell culture and mouse studies suggest NAADP analogs could help treat type 2 diabetes. In pancreatic β cells and adipocytes cultured in high glucose concentrations, NAADP increased intracellular insulin production and glucose uptake compared with no treatment. In a mouse model of obesity and type 2 diabetes, NAADP increased insulin production and glucose clearance compared with no treatment. Next steps include identifying stable NAADP analogs and testing them in additional diabetes models. SciBX 6(47); doi:10.1038/scibx.2013.1351 Published online Dec. 12, 2013	Patent applications filed; available for licensing	Park, K.-H. <i>et al. J. Biol. Chem.</i> ; published online Oct. 28, 2013; doi:10.1074/jbc.M113.489278 Contact: Uh-Hyun Kim, Chonbuk National University Medical School, Jeonju, South Korea e-mail: uhkim@chonbuk.ac.kr
Diabetes	Serotonin (5-HT _{2C}) receptor	Mouse studies suggest agonizing the 5-HT _{2C} receptor on proopiomelanocortin (POMC) neurons could help treat diabetes. In mice, knockout of the gene encoding the 5-HT _{2C} receptor specifically in POMC neurons increased diet-induced obesity, blood glucose levels and glucagon levels compared with no alteration and led to hyperinsulinemia. Conditional knockout of the gene in POMC neurons in adult mice caused the same changes in metabolic activity. Next steps include developing a 5-HT _{2C} receptor agonist that acts specifically on the neuronal subtype. SciBX 6(47); doi:10.1038/scibx.2013.1352 Published online Dec. 12, 2013	Unpatented; unavailable for licensing	Berglund, E.D. <i>et al. J. Clin. Invest.</i> ; published online Nov. 1, 2013; doi:10.1172/JCI70338 Contact: Joel K. Elmquist, The University of Texas Southwestern Medical Center, Dallas, Texas e-mail: joel.elmquist@utsouthwestern.edu Contact: Yong Xu, Baylor College of Medicine, Houston, Texas e-mail: yongx@bcm.edu
Hematology				
Myeloproliferative disorder	Retinoic acid receptor (RAR)	Mouse studies suggest RAR antagonists could help treat myelofibrosis. Mice with genetic mutations in the two receptor binding regions of nuclear receptor co-repressor 2 (NCOR2; SMRT) exhibited reduced radial bone growth and features of myelofibrosis including increased expression of fibrosis-inducing thrombopoietin (TPO) in bone marrow, which is normally repressed by the SMRT-RAR repressor complex. In irradiated mice transplanted with SMRT-mutant bone marrow that have the myelofibrosis phenotype, a RAR antagonist normalized TPO levels, bone integrity and fibrosis. Next steps could include testing RAR antagonists in additional mouse models of myeloproliferative disease. Io Therapeutics Inc. has the RAR antagonist IRX4310 in Phase I trials to treat neutropenia. SciBX 6(47); doi:10.1038/scibx.2013.1353 Published online Dec. 12, 2013	Patent and licensing status unavailable	Hong, S.-H. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Nov. 4, 2013; doi:10.1073/pnas.1318974110 Contact: Ronald M. Evans, Salk Institute for Biological Studies, La Jolla, Calif. e-mail: evans@salk.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Infectious disease				
Bacterial infection	Bacterial RNA polymerase	Computational and SAR studies suggest anthranilic acid compounds could be useful for treating Gram-positive bacterial infections. A structural model of bacterial RNA polymerase in complex with known inhibitors identified a theoretical pharmacophore predicted to have high inhibitory potency. Virtual screening and SAR studies identified anthranilic acid compounds with features corresponding to this pharmacophore. The best of these compounds showed <i>in vitro</i> potency against Gram-positive bacteria and lower rates of spontaneous resistance than the generic RNA polymerase inhibitor rifampicin. Next steps could include lead optimization and testing in animal models of bacterial infection. SciBX 6(47); doi:10.1038/scibx.2013.1354 Published online Dec. 12, 2013	Patent and licensing status undisclosed	Hinsberger, S. <i>et al. J. Med. Chem.</i> ; published online Oct. 11, 2013; doi:10.1021/jm400485e Contact: Rolf W. Hartmann, Saarland University, Saarbruecken, Germany e-mail: rolf.hartmann@helmholtz-hzi.de
HIV/AIDS	CD36 (GPIV)	<i>In vitro</i> studies suggest anti-CD36 antibodies could help treat HIV. In HIV-infected macrophages, an anti-CD36 mAb or siRNA decreased the release of HIV p24 from the macrophages, which facilitates the spread of the virus, compared with an isotype mAb or control siRNA. In HIV-infected macrophages cocultured with T cells, the anti-CD36 antibody decreased the fraction of infected T cells compared with isotype mAb. Next steps include identifying a humanized antibody against CD36. Arteria S.A. has the CD36 inhibitor AP-5258 in preclinical testing to treat dyslipidemia. SciBX 6(47); doi:10.1038/scibx.2013.1355 Published online Dec. 12, 2013	Patent application filed; licensing status unavailable	Berre, S. <i>et al. J. Exp. Med.</i> ; published online Oct. 21, 2013; doi:10.1084/jem.20130566 Contact: Philippe Benaroch, Curie Institute, Paris, France e-mail: benaroch@curie.fr
Sepsis	Ataxia telangiectasia mutated (ATM); DNA damage response	Mouse studies suggest anthracyclines could be used to treat sepsis by activating the DNA damage response. In a mouse model of severe sepsis, several anthracyclines increased survival compared with saline but did not decrease bacterial load. In the model, deletion of the DNA damage response gene <i>Atm</i> or disruption of autophagy in the lung eliminated the protective effects of anthracyclines. Next steps include conducting a clinical trial next year with epirubicin in patients with sepsis and testing anthracyclines in animal models of additional inflammatory diseases. Epirubicin is a generic topoisomerase II (TOP2) inhibitor. SciBX 6(47); doi:10.1038/scibx.2013.1356 Published online Dec. 12, 2013	Patent application filed; unavailable for licensing	Figueiredo, N. <i>et al. Immunity</i> ; published online Oct. 31, 2013; doi:10.1016/j.immuni.2013.08.039 Contact: Luis F. Moita, University of Lisbon, Lisbon, Portugal e-mail: lmoita@fm.ul.pt
<i>Staphylococcus</i>	ClpP	Cell culture and mouse studies suggest combining the antibiotic acyldepsipeptide 4 (ADEP4) with rifampicin could help eradicate biofilms. In stationary methicillin-resistant <i>S. aureus</i> (MRSA), ADEP4 activated the clpP protease and led to extensive protein degradation. In culture and a mouse model of chronic infection, ADEP4 plus rifampicin eradicated MRSA biofilms, whereas rifampicin alone did not. Next steps include additional tests on ADEP4 plus other antibiotics for toxicity and efficacy. Rifampicin is a generic antibiotic (<i>see ClpPing persistence, page 4</i>). SciBX 6(47); doi:10.1038/scibx.2013.1357 Published online Dec. 12, 2013	Patents filed covering sterilizing use of ADEP4 and related compounds in combination with other antibiotics and covering ADEP4 analogs; licensed to Arietis Corp.; available for partnering	Conlon, B.P. <i>et al. Nature</i> ; published online Nov. 13, 2013; doi:10.1038/nature12790 Contact: Kim Lewis, Northeastern University, Boston, Mass. e-mail: k.lewis@neu.edu

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Neurology				
Stroke	Thyroid hormone receptor- α	<p>Studies in human tissue and rabbits suggest thyroid hormone receptor-α agonists could protect against neurodevelopmental damage caused by intraventricular hemorrhage in preterm infants. In postmortem samples from preterm infants, brain tissue from infants with intraventricular hemorrhage had higher thyroid hormone receptor-α levels than tissue from nonhemorrhaged controls. In a rabbit pup model of intraventricular hemorrhage, thyroid hormone improved myelination and neurological function over vehicle. Next steps could include identifying and testing thyroid hormone receptor-α agonists in preclinical models of prematurity-associated neurological damage.</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1358 Published online Dec. 12, 2013</p>	Patent and licensing status undisclosed	<p>Vose, L.R. <i>et al. J. Neurosci.</i>; published online Oct. 30, 2013; doi:10.1523/JNEUROSCI.2713-13.2013 Contact: Praveen Ballabh, Maria Fareri Children's Hospital at Westchester Medical Center, Valhalla, N.Y. e-mail: pballabh@msn.com</p>
Various				
Liver disease; diabetes	Not applicable	<p><i>In vitro</i> and rat studies suggest a 2,4-dinitrophenol analog could help treat nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes. In normal rats, an analog of 2,4-dinitrophenol was less toxic than the parent compound. In rat models of NAFLD or type 2 diabetes, the analog decreased fasting plasma levels of glucose, insulin and triglycerides compared with vehicle. The compound also decreased hepatic production of very low-density lipoprotein (vLDL) and increased glucose tolerance without toxicity. Planned work includes IND-enabling studies of the analog.</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1359 Published online Dec. 12, 2013</p>	Patented; available for licensing	<p>Perry R.J. <i>et al. Cell Metab.</i>; published online Nov. 5, 2013; doi:10.1016/j.cmet.2013.10.004 Contact: Gerald I. Shulman, Yale School of Medicine, New Haven, Conn. e-mail: gerald.shulman@yale.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			
Multistage volumetric bar chart chip (MV-Chip) to detect DNA sequences	A volumetric bar chart chip could be used to detect and quantify DNA for diagnostic purposes. The chip creates a quantified ink bar chart using a three-stage, platinum film-driven reaction between catalase, which is bound to the target DNA probe, and hydrogen peroxide, which generates an oxygen signal that is amplified between platinum films and propels ink movement. The chip detected Ebola virus SNPs, target DNA diluted in complex serum and selectively detected targets in multiplexed DNA assays. Next steps include clinical validation of the chip.	Patent application filed; available for licensing	Song, Y. <i>et al.</i> <i>J. Am. Chem. Soc.</i> ; published online Oct. 25, 2013; doi:10.1021/ja4085397 Contact: Lidong Qin, Houston Methodist Research Institute, Houston, Texas e-mail: lqin@tmhs.org
SciBX 6(47); doi:10.1038/scibx.2013.1360 Published online Dec. 12, 2013			
Computational models			
Genome-based classification of lung tumors and treatments improves patient survival	Genome-based classification of lung cancer could help improve patient survival. Human lung cancers are traditionally classified based on histomorphological and immunohistochemical characteristics. Evaluation of 1,255 lung cancer specimens revealed that subtype classifications could be based on genomic data alone and mostly eliminated the large cell carcinoma subclass of tumors. Combining the genomics-based tumor classification strategy with genetically tailored cancer therapy improved survival for patients with alterations in epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) over standard chemotherapy. Next steps include conducting deep genomic analyses of small cell lung cancer in more patients and transitioning to a solely capture-based next-generation sequencing platform.	Unpatented; licensing status not applicable	The Clinical Lung Cancer Genome Project (CLCGP) and Network Genomic Medicine (NGM). <i>Sci. Transl. Med.</i> ; published online Oct. 30, 2013; doi:10.1126/scitranslmed.3006802 Contact: Roman Thomas, University of Cologne, Cologne, Germany e-mail: roman.thomas@uni-koeln.de Contact: Reinhard Büttner, same affiliation as above e-mail: reinhard.buettner@uk-koeln.de Contact: Jürgen Wolf, same affiliation as above e-mail: juergen.wolf@uk-koeln.de
SciBX 6(47); doi:10.1038/scibx.2013.1361 Published online Dec. 12, 2013			
Disease models			
Induced pluripotent stem (iPS) cell model of Phelan-McDermid syndrome (PMDS)	Studies in cell culture suggest patient-derived iPS cells could be useful for studying PMDS. PMDS is a form of autism spectrum disorder (ASD) caused by loss-of-function mutations in <i>SH3 and multiple ankyrin repeat domains 3</i> (<i>SHANK3</i> ; <i>PROSAP2</i> ; <i>SPANK-2</i>). In cell culture, iPS cell-derived neurons from patients with PMDS had lower levels of glutamate receptors and showed decreased synaptic connectivity and excitatory signaling compared with iPS cell-derived neurons from healthy individuals. In the PMDS neurons, insulin-like growth factor-1 (IGF-1) increased synaptic maturation and excitatory signaling compared with vehicle. Next steps could include identifying small molecules that mimic the effect of IGF-1 in PMDS cells. Researchers at the Icahn School of Medicine at Mount Sinai are conducting a Phase II trial of IGF-1 in PMDS.	Patent and licensing status undisclosed	Shcheglovitov, A. <i>et al.</i> <i>Nature</i> ; published online Oct. 16, 2013; doi:10.1038/nature12618 Contact: Ricardo E. Dolmetsch, Novartis Institutes for BioMedical Research, Cambridge, Mass. e-mail: ricardo.dolmetsch@novartis.com
SciBX 6(47); doi:10.1038/scibx.2013.1362 Published online Dec. 12, 2013			

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Mouse model of familial dysautonomia (FD) with neural crest lineage-specific knockout of inhibitor of κ -light polypeptide gene enhancer in B-cells kinase complex-associated protein (<i>Ikbkap</i>)	A mouse model of FD with conditional knockout of <i>Ikbkap</i> in neural crest cells could help identify new therapeutics to treat the disease. FD causes peripheral nervous system dysfunction and is caused by mutations in the <i>Ikbkap</i> gene that disrupt normal splicing. In mice, conditional knockout of <i>Ikbkap</i> in the neural crest caused early death and decreased the number of neurotrophic tyrosine kinase receptor 1 (Ntrk1; TrkA)-expressing nociceptive and thermoreceptive neurons compared with no alteration. Primary neural crest migration and development of sympathetic and dorsal root ganglia were not altered by knockout. Next steps could include using the model to test therapeutic candidates. SciBX 6(47); doi:10.1038/scibx.2013.1363 Published online Dec. 12, 2013	Patent and licensing status unavailable	George, L. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Oct. 30, 2013; doi:10.1073/pnas.1308596110 Contact: Frances Lefcort, Montana State University, Bozeman, Mont. e-mail: lefcort@montana.edu
<i>SH3</i> and multiple ankyrin repeat domains 3 (<i>Shank3</i> ; <i>Prosap2</i> ; <i>Spank-2</i>)-overexpressing mice as a model of hyperkinetic neuropsychiatric disorders	Mice overexpressing <i>Shank3</i> could provide a model of hyperkinetic neuropsychiatric disorders such as mania. <i>Shank3</i> -overexpressing, transgenic mice displayed 50% higher levels of total Shank3 protein than wild-type mice, and the protein was expressed in the same brain regions as endogenous Shank3. In open field tests, <i>Shank3</i> -overexpressing mice showed increased locomotor activity and speed compared with wild-type mice. The <i>Shank3</i> transgenic mice also displayed signs of manic behavior, including increased amphetamine-induced hyperactivity, shorter duration of immobility in tail-suspension tests, increased acoustic response and decreased prepulse inhibition. Next steps could involve testing mood-stabilizing therapies in the transgenic mice. SciBX 6(47); doi:10.1038/scibx.2013.1364 Published online Dec. 12, 2013	Unpatented; <i>Shank3</i> mice available for licensing from the Baylor College of Medicine licensing group	Han, K. <i>et al. Nature</i> ; published online Oct. 23, 2013; doi:10.1038/nature12630 Contact: Huda Y. Zoghbi, Baylor College of Medicine, Houston, Texas e-mail: hzoghibi@bcm.edu
Drug delivery			
Short RNA delivery by engineered B cells	A study in cell culture and mice suggests engineered B cells could be used to deliver therapeutic short RNAs to other immune cells. Murine B cells were harvested and transfected with a plasmid encoding an antisense RNA sequence against microRNA-150 (miR-150), which regulates T cell development. In mice, autologous transplantation of transfected B cells engineered to secrete anti-miR-150 led to knockdown of miR-150 activity in T cells. Next steps include characterizing the functional effects of miR-150 knockdown in mouse models of cancer and inflammatory disease (see Plan B for anti-miRNA, page 6). SciBX 6(47); doi:10.1038/scibx.2013.1365 Published online Dec. 12, 2013	Patent pending; available for licensing	Almanza, G. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Nov. 25, 2013; doi:10.1073/pnas.1311145110 Contact: Maurizio Zanetti, University of California, San Diego, La Jolla, Calif. e-mail: mzanetti@ucsd.edu
Staggered lamellae nanostructures for drug delivery	Staggered lamellae polyprodrug nanostructures could be useful for drug delivery. In water and dioxane mixtures, staggered lamellae structures with spiked periphery and 300 nm diameters self-assembled from amphiphilic block copolymers made up of hydrophilic poly(ethylene glycol) (PEG) and polymerized, reduction-responsive camptothecin prodrug monomers. In HeLa cells, the lamellae nanostructures were internalized faster than disk-, sphere- or flower-like polyprodrug nanostructures and released drug under reducing conditions, which mimic tumor intracellular microenvironments. In healthy rats i.v. injected with the particles, the lamellae polyprodrug nanostructures had longer half-lives than the other nanostructures. Next steps include testing the antitumor effects of the four types of self-assembled nanostructures in mouse xenograft models. SciBX 6(47); doi:10.1038/scibx.2013.1366 Published online Dec. 12, 2013	Patent application filed; available for licensing	Hu, X. <i>et al. J. Am. Chem. Soc.</i> ; published online Oct. 25, 2013; doi:10.1021/ja409686x Contact: Shiyong Liu University of Science and Technology of China, Hefei, China e-mail: sliu@ustc.edu.cn Contact: Jinming Hu, same affiliation as above e-mail: hjm85@mail.ustc.edu.cn

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Drug platforms			
Chimeric Fc γ -receptor III (CD16; FCGR3)-expressing T cells to improve antibody-dependent cell cytotoxicity (ADCC)	<p>Mouse studies suggest T cells expressing a chimeric receptor that binds to antibody Fc regions could be used to treat various cancers when combined with antibody therapies. ADCC results from the engagement of Fc γ-receptors expressed on the surface of NK cells with the Fc regions of tumor cell-bound mAbs. T cells were engineered to express a chimeric receptor made up of an FCGR3 and intracellular domains from T cell signaling molecules CD3ζ and tumor necrosis factor receptor superfamily member 9 (TNFRSF9; 4-1BB; CD137). In a mouse model of B cell lymphoma, Rituxan rituximab followed by infusion of the engineered T cells resulted in remission in all 5 mice, whereas none of 12 mice receiving engineered T cells or Rituxan alone survived. Similar results occurred in mice bearing neuroblastoma tumors that received a GD2-targeting mAb followed by the engineered T cells. Next steps include clinical trials.</p> <p>Rituxan/MabThera, an antibody against CD20 from Biogen Idec Inc. and the Genentech Inc. unit of Roche, is marketed for a variety of autoimmune diseases and hematological cancers (<i>see T cells go universal</i>, page 1).</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1367 Published online Dec. 12, 2013</p>	Patent application filed; available for licensing from the National University of Singapore and St. Jude Children's Research Hospital	Kudo, K. <i>et al. Cancer Res.</i> ; published online Nov. 6, 2013; doi:10.1158/0008-5472.CAN-13-1365 Contact: Dario Campana, National University of Singapore, Singapore e-mail: paedc@nus.edu.sg
Transcription activator-like effector nucleases (TALENs) selectively targeting individual microRNAs	<p>A library of TALEN-mediated miRNA knockouts could help study miRNA function. A library of TALEN pairs designed to disrupt 274 specific miRNA loci was designed and validated in human embryonic and leukemia cells. In breast cancer cells, TALEN pairs targeting miR-141 and miR-200C, which differ by one nucleotide in their target-selective seed regions, resulted in the upregulation of non-overlapping sets of target mRNAs and decreased cell proliferation rates compared with no TALENs. Next steps include using the TALEN library to selectively delete single miRNAs for research applications.</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1368 Published online Dec. 12, 2013</p>	Patent application filed for improved TALEN architecture; licensed by ToolGen Inc.; available for licensing	Kim, Y.-K. <i>et al. Nat. Struct. Mol. Biol.</i> ; published online Nov. 10, 2013; doi:10.1038/nsmb.2701 Contact: V. Narry Kim, Seoul National University, Seoul, South Korea e-mail: narrykim@snu.ac.kr Contact: Jin-Soo Kim, same affiliation as above e-mail: jskim01@snu.ac.kr
Imaging			
Metabotropic glutamate receptor subtype 1 (mGluR1; GRM1) binding PET radioligand for <i>in vivo</i> imaging	<p>A PET radioligand that binds mGluR1 could help diagnose and study neuropsychiatric disorders. In rhesus monkeys, injection of [¹⁸F] 4-fluoro-N-methyl-N-(4-(6-(methylamino)pyrimidin-4-yl)thiazol-2-yl)benzamide resulted in substantial brain uptake and signal distribution that matched known mGluR1 densities. In the monkeys, pretreatment with an mGluR1 antagonist quickly decreased brain radioactivity, whereas mGluR5 (GRM5) ligand pretreatment had no effect. Next steps could include testing the radioligand in humans.</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1369 Published online Dec. 12, 2013</p>	Patent and licensing status unavailable	Xu, R. <i>et al. J. Med. Chem.</i> ; published online Oct. 22, 2013; doi:10.1021/jm4012017 Contact: Victor W. Pike, National Institutes of Health (NIH), Bethesda, Md. e-mail: pikew@mail.nih.gov
Instrumentation			
Microwell displacement amplification system (MIDAS), a parallel polymerase cloning system for single-cell genome sequencing	<p>MIDAS enables massively parallel polymerase cloning, which decreases bias and improves efficiency in single-cell genome sequencing. Single cells are seeded in microarray wells in 12 nL reaction volumes, subjected to single-round amplification and extraction for library generation and DNA sequencing analyses. In proof-of-concept analyses of <i>Escherichia coli</i>, MIDAS decreased amplification bias and increased efficiency compared with conventional methods. Next steps include using the system to explore the possibility that genetic mosaicism in adult neurons influences cognitive function or neurodegenerative disease.</p> <p>SciBX 6(47); doi:10.1038/scibx.2013.1370 Published online Dec. 12, 2013</p>	Patents filed covering microwell-based amplification and the enzymatic protocol for converting DNA in the microwells to libraries; available for licensing from the University of California, San Diego Contact: Wendy Shih, University of California, San Diego Technology Transfer Office, La Jolla, Calif. e-mail: wendyshih@ucsd.edu	Gole, J. <i>et al. Nat. Biotechnol.</i> ; published online Nov. 10, 2013; doi:10.1038/nbt.2720 Contact: Kun Zhang, University of California, San Diego, La Jolla, Calif. e-mail: kzhang@bioeng.ucsd.edu

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