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By Tracey Baas, Senior Editor

A University of Maryland School of Medicine team has used a toll-like receptor 4 antagonist to treat influenza in mice.¹ The results provide a repurposing opportunity for Eisai Co. Ltd.'s toll-like receptor 4 blocker Eritoran, originally developed for sepsis, and could extend to treating respiratory infections beyond the flu.

Vaccines and antivirals—namely neuraminidase inhibitors—provide the standard of care for influenza. But the limited efficacy of vaccines together with the increasing resistance to antivirals and their short therapeutic window has spurred a need for alternative strategies.

Previous work in mice has shown that infection with influenza virus, SARS or anthrax induces the production of cellular oxidized phospholipids and other molecules that activate toll-like receptor 4 (TLR4) signaling.² This activation, which is independent of the receptor's usual ligand, lipopolysaccharide (LPS), triggers a cytokine storm that can result in acute lung injury.

Stefanie Vogel, a professor of microbiology and immunology at the University of Maryland School of Medicine, showed in 2010 that Tlr4-deficient mice could survive lethal challenge with mouse-adapted H1N1 influenza A virus, whereas wild-type mice could not.³

Based on those data, Vogel and her team hypothesized that pharmacological blockade of TLR4 might provide protection against influenza and other respiratory infections. To test this, the researchers turned to Eisai's Eritoran (E5564), a synthetic lipid A analog that failed to reduce mortality in a Phase III sepsis trial in 2011.⁴

The team included researchers from **Sigmovir Biosystems Inc.**, the **Cincinnati Children's Research Foundation**, **The University of Iowa**, the **Iowa City VA Health Care System** and Eisai's subsidiary Eisai Inc. and was co-led by Kari Ann Shirey, assistant professor of microbiology and immunology at the University of Maryland School of Medicine.

Vogel's team lethally challenged mice with a mouse-adapted H1N1 influenza A virus or a nonadapted 2009 H1N1 human pandemic influenza. In both cases, daily injection of Eritoran decreased clinical symptoms, lung pathology and cytokine signaling and increased survival compared with vehicle injection, even when started as late as six days postinfection. Eritoran also decreased lung pathology compared with vehicle in human H3N2 influenza A–infected cotton rats.

In cell culture, Eritoran did not decrease levels of virus replication, indicating that it did not directly target the virus. Instead, the team hypothesized that the compound worked by blunting the cytokine storm following infection.

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Indeed, mice infected with influenza and treated with Eritoran had substantially lower levels of oxidized phospholipids in their lungs than untreated mice. Eritoran also decreased pulmonary expression of many proinflammatory and anti-inflammatory cytokines, as well as interferon- β (IFN β ; IFN- β).

Next, the researchers looked at the effects of Eritoran on TLR4 activation. Activation of TLR4 is triggered by pathogen-induced, host-oxidized phospholipids that first bind to CD14 and then are transferred to lymphocyte antigen 96 (LY96; MD2). The lipid-MD2 complex interacts with TLR4, resulting in receptor dimerization and activation.

In Cd14-deficient mice, Eritoran did not provide protection to mice challenged with influenza. Moreover, in cell-based studies, Eritoran impeded lipid transfer from Cd14 to Md2, suggesting Eritoran's protective effects are dependent on CD14.

The team also showed that mice infected with influenza and treated with Eritoran had substantially lower levels of oxidized phospholipids in their lungs than untreated mice, suggesting that Eritoran inhibits

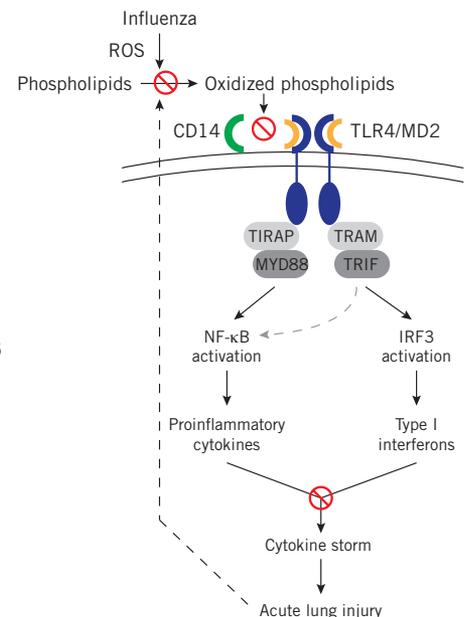
Figure 1. Proposed model of Eritoran-mediated protection in influenza infection.

Oxidized phospholipids, produced in response to viral infection, are proposed to bind to CD14 before being transferred to lymphocyte antigen 96 (LY96; MD2). The lipid-MD2 complex interacts with toll-like receptor 4 (TLR4), resulting in receptor dimerization and activation.

Intracellular adaptor proteins—toll-interleukin 1 receptor domain containing adaptor protein (TIRAP), toll-like receptor adaptor molecule 2 (TICAM2; TRAM), myeloid differentiation primary response gene 88 (MYD88) and TICAM1 (TRIF)—are then recruited, and the signal is transmitted inside the cell. TLR4 signaling that occurs through MYD88 activates NF- κ B to induce production of proinflammatory cytokines, whereas TLR4 signaling that occurs through TRIF activates interferon regulatory factor 3 (IRF3) to induce production of type I interferons.

Points in the signaling pathway at which Eritoran may block influenza virus-induced inflammation are indicated in red.

Vogel's group speculates that Eritoran can inhibit interactions of oxidized phospholipids with either CD14 or MD2, thus diminishing TLR4 signaling and production of cytokines. This would blunt the effect of cytokines on reactive oxygen species (ROS) generation and therefore limit the production of oxidized phospholipids. (Figure based on Figure 8 in ref. 1.)



oxidized phospholipid production in response to infection.

Based on the results, Vogel proposed that Eritoran blocks oxidized phospholipid-induced TLR4 signaling, which mitigates the cytokine storm and blocks further production of phospholipid oxidation induced by pathogens (see Figure 1, “Proposed model of Eritoran-mediated protection in influenza infection”).

Results were published in *Nature*.

Dennis Voelker, professor of biochemistry, molecular genetics and medicine at **National Jewish Health**, thinks the strategy provides a practical approach for dealing with the inflammatory consequences of viral infection and could help improve the overall response of the host to a virus.

Voelker is developing the antiviral and anti-inflammatory palmitoyl-oleoyl-phosphatidylglycerol (POPG) as a prophylactic and therapeutic for respiratory syncytial virus (RSV), influenza A virus, acute respiratory distress and acute lung injury.⁵⁻⁷

“Importantly, the findings provide a new route for dealing with patient populations that fail to receive immunization or that respond poorly to immunization,” Voelker said. “The approach also provides a new avenue for treating emerging influenza A strains that are resistant to neuraminidase inhibitors, which currently constitute the primary therapeutics for patients without adequate immunity to the virus.”

Vogel added that “because Eritoran has a very good safety record in people, especially very sick people, translation into the clinic would be a reasonable possibility assuming that further preclinical studies support our initial studies carried out in mice and cotton rats.”

Collecting tolls

The findings could point to a new opportunity for Eritoran and other TLR4 inhibitors, as well as for other compounds that failed in sepsis but work by stemming the cytokine storm.

“Treating sepsis is tough because you’re trying to intervene at such a late stage when the cytokine storm is extremely strong,” said Vogel. “With influenza, these drugs could help the immune system work to fight infection and blunt further initiation and potentiation of the cytokine storm.”

TLR4 inhibitors in the clinic include **Daiichi Sankyo Co. Ltd.**’s CS-4771, which is in Phase I trials to treat sepsis, and **NovImmune S.A.**’s NI-0101, a humanized mAb in Phase I testing to treat autoimmune disease and inflammation.

VBL Therapeutics Ltd. has VB-201, a TLR2 and TLR4 antagonist, in Phase II testing to treat inflammatory bowel disease (IBD) and psoriasis.

“We will continue to collaborate with Vogel’s group on preclinical research,” said Fabian Gusovsky, executive director of the chief innovation office group and special projects at Eisai Inc. and a coauthor on the *Nature* paper. “The mechanisms uncovered with our

“Because Eritoran has a very good safety record in people, especially very sick people, translation into the clinic would be a reasonable possibility assuming that further preclinical studies support our initial studies carried out in mice and cotton rats.”

—*Stefanie Vogel,*
University of Maryland School of Medicine

current work are certainly of interest, and as part of our human healthcare mission, we will continue to look at our compounds and evaluate opportunities.”

Eisai declined to discuss any patent applications.

“We want to test Eritoran in combination with standard antivirals in influenza-infected mice and cotton rats and then extend our findings to ferrets. But we are not only limited to using Eritoran. We propose to test other agents that block TLR4 or its key signaling pathways,” said Vogel.

Immediate plans for the Maryland team include testing the drug in combination with current antiviral agents, as well as in an aged

cotton rat model of influenza infection from Sigmovir Biosystems to determine the potential of Eritoran in elderly individuals who respond poorly to influenza immunizations.

“Because the drug showed efficacy against multiple strains of influenza A, the approach is likely to have broad applicability and could be useful against other viruses such as respiratory syncytial virus, for which there is no vaccine,” said Voelker.

Indeed, Vogel said, “we’ve seen some very interesting effects of Eritoran in animal models of bacterial infection and would like to be able to expand those studies as well.”

According to Vogel, the **University of Maryland, Baltimore** has filed a new use patent for the compound.

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Raising the Ras inhibitor bar

By Michael J. Haas, Senior Writer

About a year after U.S. researchers developed small molecule inhibitors of the intractable target wild-type Ras, a Japanese team has identified compounds that hit Ras mutant tumors in mice.¹ The team now is trying to ramp up the potency of the mutant-targeting compounds and optimize the structures to make them more drug-like.

Ras family proteins play central roles in cell growth and proliferation. The proteins cycle between an inactivated, GDP-bound state (Ras-GDP) and an activated, GTP-bound state (Ras-GTP). Guanine nucleotide exchange factors such as son of sevenless homolog 1 (SOS1) facilitate Ras activation by promoting the release of GDP from inactivated Ras—thereby allowing intracellular GTP to bind and reactivate it—and by enhancing the activation of Ras after it binds GTP.

Of the three Ras isoforms, K-Ras is most frequently dysregulated in cancer, with activating mutations found in about 80% of pancreatic cancers, 40% of colon cancers and 25% of lung cancers. Collectively, mutations in all three isoforms—*K-Ras*, *v-Ha-ras Harvey rat sarcoma viral oncogene homolog (HRAS)* and *neuroblastoma Ras viral oncogene (NRAS)*—occur in about 20% of all cancers.

Nevertheless, Ras proteins had long been considered undruggable because they lack well-defined surface pockets suitable for binding drug molecules. In 2012, independent groups at **Vanderbilt University School of Medicine** and the **Genentech Inc.** unit of **Roche** used fragment-based drug discovery to identify small molecules that blocked SOS1-mediated activation of wild-type K-Ras with mid-micromolar to high micromolar IC₅₀ values.²⁻⁴

Meanwhile, a Japanese team led by Tohru Kataoka had been solving the cocrystal structures of wild-type and mutant Ras proteins bound to a GTP analog. Those studies revealed a previously unreported conformation of Ras-GTP that contained potentially druggable surface pockets.⁵⁻⁷

In the new study, Kataoka's team sought to identify small molecule inhibitors of the Ras-GTP conformation.

First, the group used structure-based computational models to screen a virtual library of about 41,000 molecules for compounds that blocked interactions between Ras-GTP and one of its downstream binding partners, CRAF (RAF1). The screen yielded 97 hits, and *in vitro* testing showed that one hit—Kobe0065—had low micromolar inhibitory activity against Ras-GTP.

Next, the team conducted a computer-based search of about 160,000 compounds and identified 273 that were structurally similar to Kobe0065. One of these—Kobe2602—also had micromolar activity against Ras-GTP *in vitro*.

In human colon, pancreatic, bladder and other cancer cell lines that harbored activating Ras mutations, Kobe0065 and Kobe2602 decreased

growth compared with vehicle. Both compounds reduced growth in mutant Ras cell lines more effectively than they did in cancer cell lines expressing wild-type Ras.

In mice with xenograft tumors that harbored activating K-Ras mutations, the two compounds lowered tumor growth compared with vehicle.

Additional *in vitro* studies showed that the compounds inhibited the interactions between activated Ras and its downstream targets such as Raf proteins, MEK and phosphoinositide 3-kinase (PI3K). The compounds also blocked binding between Ras-GTP and the allosteric domain of SOS1 that accelerates Ras-GTP activation.

Kataoka is professor of molecular biology and dean of the **Kobe University Graduate School of Medicine**. The team included researchers from the **RIKEN SPring-8 Center**, the **Japan Synchrotron Radiation Research Institute** and **NEC Corp.**, which performed the computational screens and structure similarity searches.

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

“Because the compounds work according to the desired mechanism of action and show *in vitro* and *in vivo* efficacy, this study moves the field forward and raises the bar in terms

of what researchers will be able to publish on the Ras space in a good journal,” Martin Drysdale told *SciBX*. “We’re looking at this study to see if we can learn anything that applies to our own work” in targeting K-Ras with fragment-based approaches.

Drysdale is professor and head of the Drug Discovery Programme at **The Beatson Institute for Cancer Research**. Previously he was deputy research director at fragment-based and structure-based drug discovery company **Vernalis plc**.

Convergence, not competition

Kataoka said the effect of his team's compounds on downstream targets and the allosteric site of SOS1 made them clearly superior to the compounds reported by the Genentech and Vanderbilt groups. Those molecules inhibited Ras activation by blocking binding between Ras-GDP and the catalytic domain of SOS1.

Furthermore, he said, “it is doubtful whether those previously reported compounds have activity in mutant K-Ras-driven cancers because we showed in our study that inhibition of SOS1 did not alter the levels of Ras-GTP that carries activating mutations.”

Drysdale agreed that blocking Ras-GTP was preferable to blocking Ras-GDP.

“Conventional wisdom has it that something which blocks activated Ras—and most relevantly, mutated Ras—is the most obvious and sensible thing to do,” he said. “The fact that the Kataoka team's compounds do that and affect molecules downstream of Ras is interesting.”

However, he thinks it is too soon to conclude that the compounds from Kataoka's team are superior to those from the Genentech and Vanderbilt groups.

He said researchers who are developing Ras inhibitors—including his own group at Beatson—are working toward the same ultimate goal: inhibition of activated mutant Ras, which necessarily means inhibition of Ras-GTP.

“Everyone is working with what they've discovered. We're just coming at the goal from different—if somewhat overlapping—angles.”

—Martin Drysdale,
The Beatson Institute for Cancer Research

“Everyone is working with what they’ve discovered,” he said. “We’re just coming at the goal from different—if somewhat overlapping—angles.”

Indeed, Drysdale said the binding site of the Kataoka team’s compounds overlaps with the site targeted by the Genentech and Vanderbilt inhibitors. “So to say that those earlier compounds bind only to Ras-GDP is an oversimplification,” he said.

Drysdale also noted that the Kataoka team’s compounds were far from being drug-like in terms of their potency and structure. “Even the authors point this out,” he said. “Most people would not have tested these compounds at all. They are not even really good tool compounds.”

Thus, he said, the compounds identified by the Kataoka, Genentech and Vanderbilt groups are all springboards for developing future inhibitors of activated, mutant Ras.

Kataoka’s team is now conducting SAR studies on Kobe0065 and Kobe2602 to improve their potency and optimize their structures. In particular, the team wants to eliminate the compounds’ thiosemicarbazide structure because “this is generally considered to lead to cellular toxicity,” Kataoka said.

Kobe University has filed patent applications on the findings reported in *PNAS*, and the IP is available for licensing, he said.

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CRISPR model building

By Chris Cain, Senior Writer

Boston researchers have used the CRISPR-Cas9 genome modification platform to simultaneously engineer mutations into multiple genes in mice.¹ The results from the rapid, one-step process provide the best evidence to date for the potential of this method to revolutionize the creation of complex disease models.

Earlier this year, five separate teams reported on how the CRISPR (clustered, regularly interspaced short palindromic repeats)-Cas9 (CRISPR-associated protein 9) system could be adapted to engineer site-specific mutations in the genomes of mammals, bacteria and zebrafish.²⁻⁷ The method was derived from a recently identified acquired immunity-like system in bacteria, in which CRISPR-associated proteins, including Cas9, are guided by short CRISPR-encoded RNAs to cleave homologous foreign DNA contained within plasmids or bacteriophages.

Unlike other approaches that rely on large protein domains such as zinc fingers or transcription activator-like effectors (TALEs) to guide site-specific DNA editing, the specificity of Cas9 is dictated solely by DNA-RNA base pairing, greatly enhancing ease of use. Because of this, researchers and biotech executives told *SciBX* in January that the system was likely to be rapidly developed and widely used for functional genetic analysis.⁸

Now, less than four months after the proof of concept for using the method to modify a single gene at a time in cell lines was published, researchers at the **Whitehead Institute for Biomedical Research** and the **Broad Institute of MIT and Harvard** have used it to simultaneously engineer point mutations into multiple genes in a mouse.

Typically, introducing multiple gene modifications in a mouse requires several rounds of interbreeding that are both time and labor intensive.

The team, led by **Massachusetts Institute of Technology** professor of biology and Whitehead member Rudolf Jaenisch, injected one-cell mouse embryos with different concentrations of *in vitro*-transcribed mRNA encoding Cas9 along with a guide RNA targeting either *tet methylcytosine dioxygenase 1* (*Tet1*), *Tet2* or *Tet3*.

The Tet proteins convert 5-methylcytosine to 5-hydroxymethylcytosine and are being studied by many labs, including Jaenisch's, because they contribute to embryonic stem cell (ESC) pluripotency.

Using the method, the researchers were able to successfully disrupt both alleles of either *Tet1* or *Tet2* in 50%–90% of mice carried to term. For *Tet3*, only mice carrying one wild-type and one disrupted allele were born, which is consistent with previous studies that have reported that homozygous deletion of *Tet3* is lethal

to embryos.

The researchers next attempted to disrupt both *Tet1* and *Tet2* simultaneously by injecting embryos with Cas9 mRNA along with both *Tet1* and *Tet2* guide RNAs. At RNA concentrations chosen to ensure a birth rate of 25%, over half of the mice carried to term had mutations in all four alleles.

In addition, specific point mutations could be simultaneously introduced into both genes by injecting Cas9 mRNA and *Tet1* and *Tet2* guide RNAs along with single-stranded DNA oligonucleotides carrying specific mutations in *Tet1* and *Tet2*.

Finally, the team demonstrated that five genes—*Tet1*, *Tet2*, *Tet3* and the Y-chromosome-encoded *sex-determining region Y* (*Sry*) and *ubiquitously transcribed tetratricopeptide repeat* (*Uty*)—could be simultaneously targeted and disrupted in mouse ESCs.

Results were published in *Cell*.

Jaenisch said in a statement that the technology allows researchers to make mice with five mutations in about three to four weeks. In contrast, he said, conventional interbreeding of multiple, separately generated knockout mouse lines would take three to four years.

“Just as it is now much easier to make disease models, it is likewise much easier to take a patient-specific induced pluripotent stem cell line and correct the disease mutation—which has obvious therapeutic implications.”

—Kiran Musunuru,
Harvard University

Disruptive behavior

Although zinc finger nucleases (ZFNs) and TALE nucleases (TALENs) have previously been injected into embryos to create knockout strains of mammals including mice, rats and pigs, the techniques have never been used to disrupt or modify two or more different genes simultaneously.

Kiran Musunuru, assistant professor of stem cell and regenerative biology at **Harvard University**, said Cas9 editing is having a significant impact on the ease of disease model generation.

“The significantly increased efficiency that we observe with CRISPR-Cas9 in human pluripotent stem cells is making it much easier to generate disease models of all kinds. We have already found that models we were having trouble making with TALENs are now a breeze to make,” he said.

In work published in *Cell Stem Cell* last month, Musunuru used Cas9 editing to disrupt disease-associated genomic loci with an efficiency of 51%–79%.⁹ This contrasts with a study published last year by his lab in which TALENs had 0%–34% efficiency in targeting the same genes.¹⁰

When his team attempted to introduce specific point mutations into targets, the Cas9 approach was successful 11% of the time, whereas TALENs had a 1.6% success rate.

He added, “Just as it is now much easier to make disease models, it is likewise much easier to take a patient-specific induced pluripotent stem cell line and correct the disease mutation—which has obvious therapeutic implications.”

The major outstanding question for the therapeutic translation of Cas9 editing is the approach's accuracy. The *Cell* paper describes a cursory analysis of off-target effects at potential locations with sequence homology to *Tet1* or *Tet2* and found no cleavage. Nevertheless, extensive genomewide analyses of specificity have not

yet been conducted.

Musunuru said his team's work to date has shown that Cas9 can cleave genomic DNA at sites that differ from a given guide RNA by one base pair but to a much lesser degree or not at all at sites that differ by two or more base pairs.

He said another important next step will be to examine Cas9 proteins from different bacterial species, which may have varied efficiencies and specificities.

He expects studies of Cas9 specificity to advance rapidly and said studies including genomewide analysis of cleavage sites are under way by his lab and other groups. "We can expect the specificity of Cas9 to be sorted out by the end of the year," he said.

Jaenisch could not be reached for comment or to confirm the patent and licensing status of his team's findings.

Among the paper's authors was Feng Zhang, a member of the Broad Institute who led one of the first teams to describe a Cas9 editing system earlier this year and has filed a patent on the approach.

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10. Ding, Q. *et al. Cell Stem Cell* **12**, 238–251 (2013)

COMPANIES AND INSTITUTIONS MENTIONED

Broad Institute of MIT and Harvard, Cambridge, Mass.
Harvard University, Cambridge, Mass.
Massachusetts Institute of Technology, Cambridge, Mass.
Whitehead Institute for Biomedical Research, Cambridge, Mass.

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Myelination gets direct

By Lauren Martz, Staff Writer

Separate groups at the **Case Western Reserve University School of Medicine** and the **Stanford University School of Medicine** have developed similar approaches to directly reprogram rodent fibroblasts into oligodendrocyte progenitor cells.^{1,2} The direct lineage conversion method could be a safe and fast way to supply cells for myelination disorder cell therapies. Now, the teams need to show that their methods also reprogram human fibroblasts.

Myelination disorders include leukodystrophies and autoimmune conditions such as multiple sclerosis (MS). In all cases, loss of the myelin sheaths around axons of neurons impairs nerve firing and causes nervous system deficiencies.

Most available treatments for myelination disorders aim to slow demyelination or treat symptoms, but none repair or replace myelin.

One starting point for that goal is oligodendrocyte progenitor cells (OPCs), which are components of the CNS white matter that generate oligodendrocytes, the cells that produce myelin during development and after injury in the CNS.

But current sources of OPCs are limited to donor and embryonic stem cell tissues, both of which carry risks of immune rejection by the recipient. Donor OPCs also are in short supply, and cells derived from human embryonic stem cells come with a host of ethical issues and concerns about potential teratogenicity.

As an alternative, several groups including a team at Case Western have derived OPCs from rodent fibroblast-derived induced pluripotent stem (iPS) cells.³ iPS cells would be patient specific but require multiple manipulation steps, and the strategy has not yet yielded human OPCs.

The most recent approach for cell differentiation is direct lineage conversion—reprogramming somatic cells into a desired cell type—which eliminates many manipulation steps.

The Stanford team is among the groups that have successfully reprogrammed rodent fibroblasts into neurons and neural stem cells.⁴

Now, the Case Western and Stanford teams have accomplished direct lineage conversion of mouse fibroblasts into OPCs. In papers published in the same issue of *Nature Biotechnology*, the teams used forced expression of OPC-specific transcription factors to reprogram rodent fibroblasts into OPCs that generated functional, myelin-producing oligodendrocytes.

The Case Western team was led by Paul Tesar, assistant professor of genetics and genome sciences. His group transfected mouse embryonic fibroblasts with a pool of lentiviral vectors carrying eight doxycycline-inducible transcription factors that were highly expressed in OPCs and known to play roles in oligodendrocyte development.

In culture, fibroblasts were reprogrammed with the transcription factor pools into cells that expressed OPC lineage genes and suppressed

fibroblast-related genes. The cells differentiated into myelinating oligodendrocytes.

In cultured brain slices from early postnatal mice with hypomyelination due to loss of myelin basic protein (Mbp), transplantation of the reprogrammed OPCs with doxycycline led to myelination of the axons.

Transplantation of the reprogrammed cells into the dorsal spinal column of the adult mice with hypomyelination also generated Mbp-expressing myelin, suggesting the reprogrammed cells become functional oligodendrocytes *in vivo*.

The team then screened different combinations from the eight transcription factors and whittled the list of those required for reprogramming to three—*SRY-box containing gene 10 (Sox10)*, *oligodendrocyte transcription factor 2 (Olig2)* and *NK6 homeobox 2 (Nkx6-2)*.

The three transcription factors reprogrammed about 20% of the cultured mouse fibroblasts into cells with OPC properties. The OPCs could be expanded in culture for at least five passages, and the set of three transcription factors also was sufficient to reprogram mouse lung fibroblasts into OPCs.

In the other paper, Marius Wernig and colleagues at Stanford used a group of transcription factors that partially overlapped with those used by the Case Western group to reprogram both mouse and rat fibroblasts into OPCs.

Wernig is assistant professor of pathology at Stanford's Institute for Stem Cell Biology

and Regenerative Medicine.

The Stanford team first used a pool of lentiviruses carrying 10 oligodendrocyte-specific transgenes to reprogram mouse embryonic fibroblasts into OPCs. The team then shrank the cocktail to three transgenes—*Sox10*, *Olig2* and *zinc finger protein 536 (Zfp536)*—sufficient to induce effective reprogramming.

The set of three transcription factors reprogrammed rat fibroblasts with about 15% purity. The OPCs differentiated into both oligodendrocytes and astrocytes in culture.

In dorsal root ganglion neurons with extended axon beds, coculture with the reprogrammed OPCs gave rise to Mbp-expressing cells, suggesting the OPCs effectively myelinated the axons.

Wernig and colleagues used the same mouse model as the Case Western group but directly injected the reprogrammed rat cells into the corpus callosum and cerebellum brain sections of neonatal mice. Administration of doxycycline in drinking water induced sustained transgene expression and led to the formation of Mbp-expressing myelin around neurofilaments.

“Our next steps are to utilize this technology on human cells to produce patient-specific, functional oligodendrocyte progenitor cells and oligodendrocytes for use in understanding and treating human disorders of myelin,” Tesar said.

Wernig's team also plans to look into ways to convert human fibroblasts into induced OPCs.

Mike Gresser, CSO of the **Myelin Repair Foundation**, said, “These

“The most critical point for the clinical realization of the approach is the adaptation to the human system. I expect this to be doable; however, it might need a different combination of transcription factors or other stimuli such as microRNA.”

—Frank Edenhofer,
University of Bonn

human OPCs will be critical for *in vitro* studies of myelination and to evaluate myelin repair drug candidates in the human brain.”

The Myelin Repair Foundation funded the work at Case Western.

Frank Edenhofer, head of stem cell engineering at the **University of Bonn**’s Institute of Reconstructive Neurobiology, told *SciBX*, “The most critical point for the clinical

realization of the approach is the adaptation to the human system. I expect this to be doable; however, it might need a different combination of transcription factors or other stimuli such as microRNA.”

Cell source alternatives

An open question is whether the direct lineage conversion method will be better than available donor or stem cell sources.

“The key question for this approach is: What is the advantage of creating oligodendrocytes in this way specifically?” said Sheng Ding, professor of pharmaceutical chemistry at the **University of California, San Francisco** and a senior investigator at the **Gladstone Institutes**. “This is key because there are already other methods to create oligodendrocytes. We need to determine whether this would be the most practical, safe and useful method to make large masses of oligodendrocyte progenitors *in vitro* for clinical use.”

Malin Parmar, associate professor of developmental neurobiology at **Lund University**, thinks direct conversion could indeed be the optimal approach. “The advantage of direct conversion is that you bypass the pluripotent stage and thus avoid the risk of tumor formation or overgrowth due to uncontrolled proliferation after transplantation. Another advantage is that it is generally quicker and easier, which also means cheaper” than other approaches, she said. “Quicker is good because the less time cells are kept *in vitro*, the fewer things can go wrong and the fewer steps needed for quality control.”

Ding said bypassing the stem cell step does reduce the level of risk of tumorigenesis but added that “there is always a risk when transforming cells. The transformation can cause them to become tumorigenic or unstable, and you can introduce different dangers through gene modification. This still uses a gene integration method and carries the associated dangers” such as cancer or unexpected toxicities.

Both Ding and Edenhofer were concerned about the efficiency of the direct reprogramming methods.

“Based on the data reported, the conversion appears not to result in a homogeneous induced oligodendrocyte progenitor population. Instead, reprogrammed induced oligodendrocyte progenitor populations might represent a quite heterogeneous population of fully and partially reprogrammed cells,” said Edenhofer.

Ding added, “The fact that the genetic reprogramming only reprograms a small percentage of cells to the target cell type is a problem because it may not generate a functional and useful population, and

“These human OPCs will be critical for *in vitro* studies of myelination and to evaluate myelin repair drug candidates in the human brain.”

—Mike Gresser,
Myelin Repair Foundation

the contamination with cells that are not fully reprogrammed could be dangerous.”

Wernig told *SciBX* that his team is working to improve the reprogramming process to yield higher numbers of OPCs.

Therapeutic remyelination

Regardless of where the oligodendrocytes come from, the therapeutic effect in specific human diseases remains to be shown.

One concern, said Gresser, is that “inadequate quantities of oligodendrocyte progenitor cells in the brains of MS patients might not be what limits myelin repair of demyelinated lesions. It is possible that inadequate remyelination is due to factors present in or near the lesions that limit the ability of the oligodendrocyte progenitor cells that are there to proliferate and/or differentiate into myelination oligodendrocytes that properly myelinate demyelinated axons.”

He added, “It should not be taken for granted that introducing human oligodendrocyte progenitor cells into the brain of an MS patient will by itself result in good myelin repair.”

Tesar told *SciBX* that the Myelin Repair Foundation filed for a patent covering the direct cell fate conversion of somatic cells into OPCs and oligodendrocytes, which was assigned to Case Western. The IP is available for licensing.

Wernig said that two years ago, Stanford filed a patent application for the direct conversion of fibroblasts to neurons with a possibility to also induce oligodendrocytes using the same methods. He said that for undisclosed reasons, a patent was never issued.

Martz, L. *SciBX* 6(19); doi:10.1038/scibx.2013.456
Published online May 16, 2013

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Contact: Marius Wernig, Stanford University School of Medicine, Stanford, Calif.
e-mail: wernig@stanford.edu
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Case Western Reserve University School of Medicine, Cleveland, Ohio
Gladstone Institutes, San Francisco, Calif.
Lund University, Lund, Sweden
Myelin Repair Foundation, Saratoga, Calif.
Stanford University School of Medicine, Stanford, Calif.
University of Bonn, Bonn, Germany
University of California, San Francisco, Calif.

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 | | | | |
| Autoimmune disease | IL-7; IL-7 receptor (IL-7R; CD127) | <i>In vitro</i> and mouse studies suggest soluble IL-7R can enhance the activity of IL-7, a cytokine that plays roles in autoimmune disease and antiviral immunity. In cultured T cells, soluble IL-7R plus IL-7 increased the duration of IL-7 activity compared with IL-7 alone. In a mouse model for experimental autoimmune encephalitis (EAE), IL-7 plus soluble IL-7R exacerbated disease pathology. Next steps include evaluating soluble IL-7R as a biomarker for multiple sclerosis (MS) and using soluble IL-7R to enhance the antiviral effect of IL-7 in models for infection. Cytheris S.A.'s CYT107 recombinant IL-7 is in Phase II trials to treat progressive multifocal leukoencephalopathy (PML). | Unpatented; licensing status not applicable | Lundström, W. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online April 22, 2013; doi:10.1073/pnas.1222303110 Contact: Crystal L. Mackall, National Cancer Institute, Bethesda, Md. e-mail: mackallc@mail.nih.gov |
| SciBX 6(19); doi:10.1038/scibx.2013.457 Published online May 16, 2013 | | | | |
| Cancer | | | | |
| Acute myelogenous leukemia (AML) | Hemopoietic cell kinase (HCK) | SAR and mouse studies suggest HCK inhibitors could be useful for treating AML. SAR studies identified an HCK inhibitor with higher specificity for HCK than for related kinases. In primary cultured AML cells, the compound inhibited tumor growth with nanomolar IC ₅₀ values. In a mouse xenograft model for human AML, the compound prevented tumor growth in bone and spleen. Next steps include preclinical toxicology, pharmacokinetic and pharmacodynamic studies. | Patent pending; available for licensing | Saito, Y. <i>et al. Sci. Transl. Med.</i> ; published online April 17, 2013; doi:10.1126/scitranslmed.3004387 Contact: Fumihiko Ishikawa, RIKEN Research Center for Allergy and Immunology, Yokohama, Japan e-mail: f_ishika@rcai.riken.jp |
| SciBX 6(19); doi:10.1038/scibx.2013.458 Published online May 16, 2013 | | | | |
| Brain cancer | Dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A (DYRK1A); epidermal growth factor receptor (EGFR) | Cell culture and mouse studies suggest inhibiting DYRK1A could help treat glioblastoma. In patient glioblastoma samples, increased expression of EGFR, a common driver of the disease, correlated with increased expression of DYRK1A. In cultured glioblastoma cells, <i>DYRK1A</i> -targeting small hairpin RNA or a nonspecific DYRK1A inhibitor both decreased EGFR levels and the number of tumor-initiating cells compared with control shRNA or vehicle, respectively. In a mouse xenograft model for glioblastoma, shRNA-mediated knockdown of DYRK1A led to decreased tumor growth compared with no knockdown. Next steps include testing DYRK1A inhibition in combination with chemotherapy and radiotherapy and testing more specific DYRK1A inhibitors. | Unpatented; licensing status not applicable | Pozo, N. <i>et al. J. Clin. Invest.</i> ; published online May 1, 2013; doi:10.1172/JCI63623 Contact: Pilar Sánchez-Gómez, Carlos III Health Institute, Majadahonda, Spain e-mail: psanchezg@isciii.es |
| SciBX 6(19); doi:10.1038/scibx.2013.459 Published online May 16, 2013 | | | | |

This week in therapeutics (continued)

| Indication | Target/marker/ pathway | Summary | Licensing status | Publication and contact information |
|---------------|--|--|--|--|
| Breast cancer | β -Catenin (CTNNB1); HER2 (EGFR2; ErbB2; neu); p21 protein (Cdc42 Rac)-activated kinase 1 (PAK1) | <i>In vitro</i> and mouse studies suggest inhibiting PAK1 could help treat HER2 ⁺ breast cancers. In HER2 ⁺ breast cancer cells, small hairpin RNA-mediated knockdown of PAK1 decreased cell proliferation and CTNNB1 levels compared with PAK2 knockdown. In mouse xenograft models for HER2 ⁺ human breast cancer, combined small molecule-mediated inhibition of PAK1 and CTNNB1 signaling caused more potent tumor growth prevention than inhibition of either target alone. In breast cancer cells resistant to the HER2 inhibitor Herceptin trastuzumab, blockade of PAK1 and CTNNB1 restored sensitivity to the drug. Next steps include clinical trials of PAK1 inhibitors that show favorable bioavailability. Roche's Genentech Inc. unit and Chugai Pharmaceutical Co. Ltd. market Herceptin to treat breast and gastric cancers. Marina Biotech Inc.'s CEQ508, an oral RNAi targeting CTNNB1, is in Phase I/II testing to treat colorectal cancers. Prism Pharma Co. Ltd. and Eisai Co. Ltd. have PRI-724, a CTNNB1 inhibitor, in Phase I testing to treat solid tumors. SciBX 6(19); doi:10.1038/scibx.2013.460 Published online May 16, 2013 | Findings unpatented; mouse models for evaluating Pak1 loss available for licensing | Arias-Romero, L.E. <i>et al. Cancer Res.</i> ; published online April 10, 2013; doi:10.1158/0008-5472.CAN-12-4453 Contact: Jonathan Chernoff, Fox Chase Cancer Center, Philadelphia, Pa. e-mail: j_chernoff@fccu.edu |
| Breast cancer | Discoidin domain receptor tyrosine kinase 2 (DDR2) | <i>In vitro</i> and mouse studies suggest inhibiting DDR2 could help treat metastatic breast cancer. In primary invasive breast tumors, DDR2 expression was greater than that in normal breast tissue and correlated with reduced survival. In a human breast cancer cell line, small hairpin RNA against DDR2 decreased invasion and migration compared with a scrambled control shRNA. In normal mice, implantation of a mouse breast cancer cell line pretreated with shRNA against <i>Ddr2</i> decreased the number and size of lung metastases compared with implantation of cells pretreated with a scrambled control shRNA. Ongoing work includes a computational screen that identifies small molecule DDR2 inhibitors. SciBX 6(19); doi:10.1038/scibx.2013.461 Published online May 16, 2013 | Unpatented; available for licensing or partnering | Zhang, K. <i>et al. Nat. Cell Biol.</i> ; published online May 5, 2013; doi:10.1038/ncb2743 Contact: Gregory D. Longmore, Washington University in St. Louis, St. Louis, Mo. e-mail: glongmor@dom.wustl.edu |
| Cancer | DNA | <i>In vitro</i> and mouse studies suggest prodrugs of the duocarmycin family of DNA-alkylating agents could help treat cancer. In a murine leukemia cell line, two of the lead <i>N</i> -acyl <i>O</i> -amino prodrugs of duocarmycin analogs showed cytotoxicity with subnanomolar IC ₅₀ values. In a mouse model for leukemia, one of the lead prodrugs increased survival compared with the nonprodrug compound. Ongoing work includes testing the lead compound in rodent xenograft models for undisclosed cancers. Synthon B.V.'s anti-HER2-ADC, an antibody-drug conjugate consisting of trastuzumab conjugated to duocarmycin analogs using the company's SpaceLink technology, is in preclinical testing to treat cancer. Roche's Genentech Inc. unit and Chugai Pharmaceutical Co. Ltd. market Herceptin trastuzumab, an anti-HER2 (EGFR2; ErbB2; neu) antibody, to treat breast and gastric cancers. SciBX 6(19); doi:10.1038/scibx.2013.462 Published online May 16, 2013 | Patented; unlicensed | Wolfe, A.L. <i>et al. J. Med. Chem.</i> ; published online April 29, 2013; doi:10.1021/jm400413r Contact: Dale L. Boger, The Scripps Research Institute, La Jolla, Calif. e-mail: boger@scripps.edu |

This week in therapeutics (continued)

| Indication | Target/marker/pathway | Summary | Licensing status | Publication and contact information |
|------------|---|--|---|---|
| Cancer | Enhancer of zeste homolog 2 (EZH2); SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily b member 1 (SMARCB1; SNF5) | Cell culture and mouse studies identified an EZH2 inhibitor that could help treat <i>SMARCB1</i> mutant malignant rhabdoid tumors (MRTs). In <i>SMARCB1</i> mutant MRT cells, the EZH2 inhibitor EPZ-6438 blocked proliferation with nanomolar IC ₅₀ values. In mouse xenograft models for <i>SMARCB1</i> mutant MRT, oral EPZ-6438 for 21 days caused tumor regression without regrowth 32 days after inhibitor cessation. Next steps could include testing the EZH2 inhibitor in additional models for cancer. Epizyme Inc. and Eisai Co. Ltd. have EPZ-6438 in preclinical testing to treat lymphoma. SciBX 6(19); doi:10.1038/scibx.2013.463 Published online May 16, 2013 | Patent and licensing status unavailable | Knutson, S.K. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online April 25, 2013; doi:10.1073/pnas.1303800110 Contact: Heike Keilhack, Epizyme Inc., Cambridge, Mass. e-mail: hkeilhack@epizyme.com |
| Cancer | Insulin receptor substrate 1 (IRS1); phosphatidylinositol 3-kinase catalytic subunit α -polypeptide (PIK3CA; p110 α); phosphoinositide 3-kinase- α (PI3K α) | Cell culture and mouse studies suggest disrupting the interaction between IRS1 and mutant p110 α could help treat cancer. p110 α is the catalytic subunit of PI3K α . In a human colorectal cancer cell line, E545K mutant p110 α interacted with IRS1, whereas wild-type p110 α did not. In a mouse xenograft model for human colorectal cancer that expressed the E545K mutant p110 α , injection of a stapled peptide that disrupts the IRS1–mutant p110 α interaction decreased tumor growth compared with injection of a control peptide or vehicle. Next steps include developing peptidomimetics with improved pharmacokinetics and potency and developing an assay to screen for small molecules that could disrupt the IRS1–mutant p110 α interaction. Gene Signal International S.A.'s aganirsen, an antisense oligonucleotide that targets IRS1 mRNA, is in preclinical development to treat bladder cancer. At least four companies have PI3K α -specific inhibitors in Phase I or Phase II testing for cancer. SciBX 6(19); doi:10.1038/scibx.2013.464 Published online May 16, 2013 | Patent application filed; available for licensing from the Case Western Reserve University Technology Transfer Office | Hao, Y. <i>et al. Cancer Cell</i> ; published online May 13, 2013; doi:10.1016/j.ccr.2013.03.021 Contact: Zhenghe Wang, Case Western Reserve University, Cleveland, Ohio e-mail: zxw22@case.edu Contact: Weiping Zheng, Jiangsu University School of Pharmacy, Zhenjiang, China e-mail: wzheng@ujs.edu.cn |
| Cancer | K-Ras; son of sevenless homolog 1 (SOS1); CRAF (RAF1) | <i>In vitro</i> , cell culture and mouse studies identified small molecule K-Ras inhibitors that could help treat cancer. Previous studies identified small molecules that bound to K-Ras and blocked its activation by SOS1. In the current study, structure-based drug discovery identified a new series of compounds that inhibited the interaction between GTP-bound K-Ras, RAF1 and SOS1. In mice with colon cancer cells driven by oncogenic K-Ras, the compounds decreased tumor growth compared with vehicle. Next steps include optimizing structures of the compounds and improving their potency. Roche's Genentech Inc. has a discovery stage K-Ras inhibitor program (<i>see Raising the Ras inhibitor bar, page 4</i>). SciBX 6(19); doi:10.1038/scibx.2013.465 Published online May 16, 2013 | Patent application filed; available for licensing | Shima, F. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online April 29, 2013; doi:10.1073/pnas.1217730110 Contact: Tohru Kataoka, Kobe University Graduate School of Medicine, Kobe, Japan e-mail: ataoka@people.kobe-u.ac.jp Contact: Fumi Shima, same affiliation as above e-mail: sfumi@med.kobe-u.ac.jp |
| Cancer | Toll-like receptor 5 (TLR5) | Mouse studies suggest liver-targeted TLR5 agonists could help prevent cancer metastasis to that organ and radiation-induced damage to healthy tissues. In mouse models for metastatic cancer, the TLR5 agonist CBLB502 inhibited growth of tumor cells in the liver and decreased radiation-induced death of hematopoietic progenitor cells compared with saline. In the model, blocking the liver's blood supply inhibited the radioprotective effect of CBLB502, suggesting the liver is the key mediator of the TLR5-dependent effects. Next steps could include designing liver-targeted TLR5 agonists. Cleveland BioLabs Inc., which provided funding for the study, has CBLB502 in Phase I testing to treat various cancers. The compound has completed Phase IIa testing to treat acute radiation syndrome. SciBX 6(19); doi:10.1038/scibx.2013.466 Published online May 16, 2013 | Patent and licensing status unavailable | Burdelya, L.G. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online April 29, 2013; doi:10.1073/pnas.1222805110 Contact: Andrei V. Gudkov, Roswell Park Cancer Institute, Buffalo, N.Y. e-mail: andrei.gudkov@roswellpark.org |

This week in therapeutics (continued)

| Indication | Target/marker/ pathway | Summary | Licensing status | Publication and contact information |
|---------------------------|--|--|--|--|
| Multiple myeloma (MM) | Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6; NCA; CD66c) | Cell culture studies suggest inhibiting CEACAM6 could help improve immunotherapy treatments for MM. In the study, T cells cultured with MM cells showed comparable reactivity and cytotoxicity to T cells cultured alone. In the cell lines and in patient samples, an anti-CEACAM6 mAb or small interfering RNA against the antigen restored T cell reactivity and cytotoxicity against MM cells, whereas control mAbs or siRNA did not. Next steps could include evaluating an anti-CEACAM6 mAb in combination with T cell immunotherapy in MM models. At least three companies have CEACAM6-targeting antibodies in preclinical development to treat various solid cancers. SciBX 6(19); doi:10.1038/scibx.2013.467 Published online May 16, 2013 | Patent and licensing status unavailable | Witzens-Harig, M. <i>et al. Blood</i> ; published online April 19, 2013; doi:10.1182/blood-2012-05-429415 Contact: Philipp Beckhove, German Cancer Research Center, Heidelberg, Germany e-mail: p.beckhove@dkfz.de |
| Hepatic disease | | | | |
| Liver fibrosis | Mothers against decapentaplegic homolog 3 (MADH3; SMAD3); transforming growth factor receptor-β1 (TGFB1); vitamin D receptor (VDR) | <i>In vitro</i> and mouse studies suggest VDR agonists could help treat or prevent liver fibrosis. In mice, prophylactic and therapeutic use of the VDR agonist calcipotriol prevented or decreased chemically induced liver fibrosis compared with no treatment. In primary hepatic stellate cells, VDR agonism suppressed TGFB1-induced activation of profibrotic SMAD3 genes. Next steps include using cell- or tissue-targeting approaches to develop vitamin D-based therapeutics. Leo Pharma A/S and CSL Ltd. market Dovonex calcipotriol/betamethasone to treat psoriasis. At least four other companies have vitamin D analogs in Phase II testing or earlier to treat cancer or autoimmune diseases. SciBX 6(19); doi:10.1038/scibx.2013.468 Published online May 16, 2013 | Patent application filed; unavailable for licensing | Ding, N. <i>et al. Cell</i> ; published online April 25, 2013; doi:10.1016/j.cell.2013.03.028 Contact: Ronald M. Evans, Salk Institute for Biological Studies, La Jolla, Calif. e-mail: evans@salk.edu Contact: Michael Downes, same affiliation as above e-mail: downes@salk.edu |
| Infectious disease | | | | |
| Influenza virus | Toll-like receptor 4 (TLR4) | Rodent studies suggest a lipid A analog that blocks TLR4 activation could help treat influenza virus infection. In mice challenged with a lethal dose of influenza virus, the synthetic lipid A analog Eritoran decreased disease pathology and cytokine signaling compared with saline. Eritoran also increased survival, even when started as late as six days postinfection. In cotton rats, Eritoran also decreased lung pathology and cytokine signaling compared with saline. Next steps include testing the compound in aged cotton rat models of influenza infection and in combination with antivirals. Eisai Co. Ltd.'s Eritoran (E5564), a synthetic lipid A analog that blocks TLR4 activation, failed to meet the primary endpoint of reduced mortality in Phase III testing for sepsis, and development has been put on hold (<i>see Eritoran insight for influenza treatment, page 1</i>). SciBX 6(19); doi:10.1038/scibx.2013.469 Published online May 16, 2013 | Patent application filed covering method of use; available for licensing | Shirey, K.A. <i>et al. Nature</i> ; published online May 1, 2013; doi:10.1038/nature12118 Contact: Stefanie N. Vogel, University of Maryland School of Medicine, Baltimore, Md. e-mail: svogel@som.umaryland.edu |
| Leishmaniasis | Not applicable | <i>In vitro</i> and hamster studies suggest new classes of quinazolinone analogs could help treat leishmaniasis. Chemical synthesis and <i>in vitro</i> testing identified multiple lead compounds, including two quinazolinone-triazine hybrids and a quinazolinone peptide. Those molecules inhibited replication of <i>Leishmania donovani</i> with IC ₅₀ values in the low micromolar or high nanomolar range. In a hamster model for leishmaniasis, selected lead compounds inhibited <i>L. donovani</i> replication by over 50% without causing observable toxicity. Future studies could include optimizing the lead compounds. SciBX 6(19); doi:10.1038/scibx.2013.470 Published online May 16, 2013 | Patent and licensing status unavailable | Sharma, M. <i>et al. J. Med. Chem.</i> ; published online April 23, 2013; doi:10.1021/jm400053v Contact: Prem M.S. Chauhan, CSIR-Central Drug Research Institute, Lucknow, India e-mail: prem_chauhan_2000@yahoo.com |

This week in therapeutics (continued)

| Indication | Target/marker/ pathway | Summary | Licensing status | Publication and contact information |
|--------------------------|-------------------------------|---|---|---|
| Viral infection | Not applicable | <p><i>In vitro</i> and mouse studies identified membrane-intercalating photosensitizers that could help treat viral infections. <i>In vitro</i> studies showed that the previously identified viral fusion inhibitor LJ001 is a light-activated, membrane-intercalating photosensitizer that catalyzes the oxidation of unsaturated phospholipids in viral membranes. Follow-up SAR studies led to the identification of oxazolidine-2,4-dithiones with 100-fold increases for <i>in vitro</i> potency and 10- to 100-fold increases in bioavailability over LJ001. In mice challenged with a lethal dose of Rift Valley fever virus, two of the three lead oxazolidine-2,4-dithiones increased survival times compared with no treatment. Next steps could include testing the lead oxazolidine-2,4-dithiones in additional models for viral infection (<i>see</i> Lou, K.-J., <i>SciBX</i> 3(8); doi:10.1038/scibx.2010.235).</p> <p>SciBX 619; doi:10.1038/scibx.2013.471 Published online May 16, 2013</p> | Patent application filed; licensing status unavailable | Vigant, F. <i>et al. PLoS Pathog.</i> ; published online April 18, 2013; doi:10.1371/journal.ppat.1003297 Contact: Benhur Lee, University of California, Los Angeles, Calif. e-mail: blebhl@ucla.edu |
| Neurology | | | | |
| Alzheimer's disease (AD) | β -Amyloid (A β) | <p>Mouse studies suggest a conjugate vaccine against the amino-terminal fragment of Aβ could help prevent AD. The vaccine consists of residues 1–15 of Aβ conjugated to a diphtheria toxoid-derived carrier and formulated in an oil-based nanoparticle emulsion. In a mouse model for AD, the vaccine induced an antibody response and an anti-inflammatory pattern of T cell activity, and it decreased histological and cognitive abnormalities compared with vehicle control. Next steps include studies in aged mouse AD models and preclinical development of the vaccine candidate.</p> <p>Researchers at Mercia Pharma Inc. are coauthors on the study, and the company has an AD vaccine candidate in preclinical development.</p> <p>At least 11 other companies have vaccines for AD in Phase II testing or earlier development.</p> <p>SciBX 6(19); doi:10.1038/scibx.2013.472 Published online May 16, 2013</p> | Patents filed by Mercia Pharma on vaccine formulation; available for partnering | Liu, B. <i>et al. J. Neurosci.</i> ; published online April 17, 2013; doi:10.1523/JNEUROSCI.5924-12.2013 Contact: Cynthia A. Lemere, Brigham and Women's Hospital and Harvard Medical School, Boston, Mass. e-mail: clemere@rics.bwh.harvard.edu |

This week in techniques

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

This week in techniques includes findings about research tools, disease models and manufacturing processes that have the potential to enable or improve all stages of drug discovery and development.

| Approach | Summary | Licensing status | Publication and contact information |
|---|---|---|---|
| Assays & screens | | | |
| Single-molecule analysis of epigenetic markers | <p>Single-molecule analysis of epigenetic markers could help identify epigenome-based cancer biomarkers and monitor the effects of epigenome-modifying drugs. Existing approaches, including chromatin immunoprecipitation and bisulfite sequencing, only allow the direct identification of one marker at a time and a second one by association but not by direct observation. The single-molecule approach uses fluorescently labeled antibodies to target a specific epigenetic mark on intact chromatin and a microfluidic device to separate and detect the fluorescently labeled chromatin complexes. The technique was used to detect two epigenetic markers simultaneously and monitor their methylation levels following treatment with a DNA methyltransferase inhibitor. Next steps could include further refining the technique to enable simultaneous identification of three or more epigenetic markers.</p> <p>SciBX 6(19); doi:10.1038/scibx.2013.473 Published online May 16, 2013</p> | Patent and licensing status undisclosed | <p>Murphy, P.J. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online April 22, 2013; doi:10.1073/pnas.1218495110 Contact: Paul D. Soloway, Cornell University, Ithaca, N.Y. e-mail: soloway@cornell.edu Contact: Harold G. Craighead, same affiliation as above e-mail: hgc1@cornell.edu</p> |
| Disease models | | | |
| An aged mouse model for protein tyrosine phosphatase non-receptor type 22 (PTPN22; LYP)-associated autoimmunity | <p>An aged mouse model for LYP-associated autoimmune diseases could be used to identify new treatments for conditions including multiple sclerosis (MS) and rheumatoid arthritis (RA). In mice engineered to express PEP-R619W, the mouse ortholog of a human LYP variant that is associated with autoimmune disease, aged mice developed autoantibodies and features of systemic autoimmunity, whereas young mice did not. In the aged mouse model, the stability of the variant protein was similar to that of wild-type protein, but it was functionally altered and caused increased T and B cell proliferation. Next steps could include using the model to identify new autoimmune disease therapeutics.</p> <p>SciBX 6(19); doi:10.1038/scibx.2013.474 Published online May 16, 2013</p> | Patent and licensing status unavailable | <p>Dai, X. <i>et al. J. Clin. Invest.</i>; published online April 24, 2013; doi:10.1172/JCI66963 Contact: David J. Rawlings, Seattle Children's Research Institute, Seattle, Wash. e-mail: drawling@u.washington.edu</p> |
| Bioluminescent mice to monitor progression of muscular dystrophy | <p>Mice with inducible luciferase expression in muscle stem cells could help identify new therapeutic candidates to treat muscular dystrophies. In the mice, muscle injury led to increased luciferase expression compared with baseline, and muscle recovery led to a return to baseline luciferase expression. In a mouse model for limb-girdle muscular dystrophy 2B that also had inducible luciferase expression in muscle stem cells, disease progression could be monitored via luciferase expression, which also correlated with the expression of conventional disease markers. Next steps could include using the model to monitor the effect of therapeutic candidates on muscular dystrophy progression.</p> <p>SciBX 6(19); doi:10.1038/scibx.2013.475 Published online May 16, 2013</p> | Patent and licensing status unavailable | <p>Maguire, K.K. <i>et al. J. Clin. Invest.</i>; published online April 24, 2013; doi:10.1172/JCI68458 Contact: Thomas A. Rando, Stanford University, Stanford, Calif. e-mail: rando@stanford.edu</p> |

This week in techniques (continued)

| Approach | Summary | Licensing status | Publication and contact information |
|--|---|--|---|
| Markers | | | |
| Brain metastasis markers in epithelial cell adhesion molecule (EpCAM) ⁻ circulating tumor cells (CTCs) | <p>A protein signature specific to EpCAM⁻ CTCs could be used for early detection of brain metastasis in patients with breast cancer. EpCAM⁻ CTCs were isolated from patients with breast cancer, and a subset of the cells that overexpressed <i>HER2</i> (<i>EGFR2</i>; <i>ErbB2</i>; <i>neu</i>), <i>epidermal growth factor receptor</i> (<i>EGFR</i>), <i>heparanase</i> (<i>HPSE</i>) and <i>Notch 1</i> (<i>NOTCH1</i>) was used to generate stable cell lines. In immunodeficient mice, injection of cells taken from such cell lines led to brain metastases in 60%–80% of the animals, whereas only 0%–20% of those injected with CTCs that did not express the protein signature had brain metastases. Researchers did not disclose next steps, which could include confirming the signature in larger patient populations and determining the therapeutic potential of targeting HPSE, which has previously been associated with brain metastasis.</p> <p>SciBX 6(19); doi:10.1038/scibx.2013.476 Published online May 16, 2013</p> | Patent and licensing status undisclosed | <p>Zhang, L. <i>et al. Sci. Transl. Med.</i>; published online April 10, 2013; doi:10.1126/scitranslmed.3005109 Contact: Dario Marchetti, Baylor College of Medicine, Houston, Texas e-mail: marchett@bcm.edu</p> |
| Using integrin α_2 (VLA-2; CD49B) and lymphocyte-activation gene 3 (LAG3; CD223) to isolate type 1 T _{reg} cells | <p>CD49B and LAG3 could be useful for identifying and isolating populations of type 1 T_{reg} cells, which promote and maintain immune tolerance. In both mouse and human type 1 T_{reg} cells, expression profiling showed that CD49B and LAG3 were stably and selectively coexpressed on the cell surface. Fluorescence-activated cell sorting (FACS) isolated T cell populations enriched for CD49B⁺ and LAG3⁺ cells. In culture, the enriched T cell population had greater immunosuppressive capacity and IL-10 secretion than the original, unenriched T cell population. Next steps include comparing the activity of type 1 T_{reg} cells from healthy subjects and patients who have autoimmune diseases and developing clinical-grade protocols to isolate and purify such cells.</p> <p>SciBX 6(19); doi:10.1038/scibx.2013.477 Published online May 16, 2013</p> | <p>Provisional patent application filed; available for licensing from the San Raffaele Scientific Institute Office of Biotechnology Transfer Contact: Paola Pozzi, San Raffaele Scientific Institute, Milan, Italy e-mail: pozzi.paola@hsr.it</p> | <p>Gagliani, N. <i>et al. Nat. Med.</i>; published online April 28, 2013; doi:10.1038/nm.3179 Contact: Maria-Grazia Roncarolo, San Raffaele Scientific Institute, Milan, Italy e-mail: m.roncarolo@hsr.it Contact: Richard A. Flavell, Yale University, New Haven, Conn. e-mail: richard.flavell@yale.edu</p> |

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