

THIS WEEK**ANALYSIS****COVER STORY****1 Building meditope-enabled mAbs**

Researchers at the Beckman Research Institute at City of Hope have provided proof of concept for adapting the functionality of mAbs by modifying them with engineered peptides called meditopes. The institute founded Meditope Biosciences to commercialize the technology.

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7 Benztropine for MS

Although candidate compounds to treat secondary progressive multiple sclerosis abound, few lead to neuronal remyelination. A new study has suggested that an existing Parkinson's disease drug—benztropine—or a derivative could accomplish the task.

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A multi-institute team collaborating with the FDA has determined why patients with a form of severe hemophilia rarely develop neutralizing antibodies against factor VIII replacement therapy. The group is developing an algorithm to predict the likelihood of antibody development against other recombinant protein-based therapies.

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By Kai-Jye Lou, Senior Writer

Linker technologies for generating antibody-drug conjugates typically require a labor-intensive process of reengineering the antibody for every application. Researchers at the **Beckman Research Institute at City of Hope** have published proof-of-concept data on a platform to generate mAb conjugates with other molecules of interest that eliminates the need to reengineer the antibody for each conjugate.¹

Institute spinout **Meditope Biosciences Inc.** is commercializing the technology, which involves building a site into mAbs that can bind to peptides called meditopes that serve as linkers to various payloads. The startup raised \$3.6 million in a series A round in July and is seeking industry partnerships.

Antibody-drug conjugate (ADC) linkers can disrupt a mAb's native properties in an undesirable manner—for example, by decreasing antigen-binding affinity, specificity and stability or increasing immunogenicity.²

Thus, researchers need to reassess and likely reoptimize the properties of a mAb each time they reengineer it to carry a different molecule. Doing so is labor intensive and can become cost prohibitive when generating a series of candidates.

A research group led by John Williams has been studying the structure of various antibodies in order to develop a more efficient approach to link compounds to mAbs. Williams is director of the X-ray Crystallography Core Facility, co-director of the Drug Discovery and Structural Biology Core and an associate professor in the Department of Molecular Medicine at the Beckman Research Institute.

In a City of Hope press release in June, Williams' team announced the chance discovery of a peptide-binding site unique to Erbitux cetuximab and then showed that a peptide called a meditope could bind to the site.

Williams' group hypothesized that it might be possible to use the site and meditopes as a convenient means to attach different compounds to a mAb by first conjugating a compound to a meditope.

Eli Lilly and Co., Bristol-Myers Squibb Co. and Merck KGaA market Erbitux, a chimeric antibody against epidermal growth factor receptor (EGFR), to treat head and neck cancer and colorectal cancer.

Now, the City of Hope researchers have characterized the binding site along with its interaction with the meditope peptide and have shown proof of concept on how to exploit it (*see* Figure 1, "Meditope-enabled mAbs").

Analysis of the crystal structure of the Erbitux Fab domain in complex with meditope peptides showed that the meditope-binding site

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is located within a cavity formed by the mAb's light and heavy chains. Follow-up biophysical and sequencing studies identified the key amino acid residues that mediate mediotope binding. The studies also showed that binding of the mediotope to the mAb occurs via a noncovalent interaction that does not disrupt the mAb's ability to bind to its target antigen.

With the structural and sequence data in hand, the researchers were then able to engineer the mediotope-binding site into another marketed mAb—the humanized anti-HER2 (EGFR2; ErbB2; neu) mAb Herceptin trastuzumab. Importantly, creating the binding site and binding a mediotope to it did not significantly disrupt the ability of the mediotope-enabled trastuzumab to bind its target antigen.

However, the researchers found that monovalent mediotopes only bound to mAbs with moderate affinity and could be removed with a wash procedure—an issue that could preclude their potential use for attaching imaging agents and therapeutic payloads to the mAb.

Thus, the researchers engineered a bivalent mediotope-Fc construct and showed that it could bind to Erbitux and the mediotope-enabled Herceptin with high enough affinity to resist an extensive washing procedure without significantly disrupting native antigen binding.

Data were published in the *Proceedings of the National Academy of Sciences*. Roche's Genentech Inc. unit markets Herceptin to treat breast and gastric cancers.

"The strategy presented in the report clearly offers a novel mAb linker technology platform, where any mAb could easily be turned into a site-specific, Lego-like system that is able to attach small molecules or toxins to antibodies without the need for chemical conjugation," said Iqbal Grewal, CSO at ImmunGene Inc.

"If you're trying to make an antibody-drug conjugate, current methods generally involve chemically locking a payload to the antibody. But doing this can disrupt the antibody's native properties, such as antigen binding," added Williams, the corresponding author and a cofounder of Mediotope. "What we have developed is a system to hitch a molecule onto an antibody via a noncovalent interaction that does not interfere with antigen binding."

Williams said that the mediotope platform could have utility across a range of settings, including the development of new ADCs or reagents for antibody-pretargeted imaging and immunoprecipitation assays.

Grewal said that bivalent mediotopes also could be prepared and that these could be used to cross-link mediotope-enabled mAbs to improve the efficacy or signaling properties of the antibodies. ImmunGene is developing mAbs fused to immune effector molecules to treat various types of cancer.

Mediotope benefits

Williams said that the mediotope platform obviates the need to reengineer a mediotope-enabled mAb every time the user wants to attach a different

"What we have developed is a system to hitch a molecule onto an antibody via a noncovalent interaction that does not interfere with antigen binding."

**—John Williams,
Beckman Research Institute
at City of Hope**

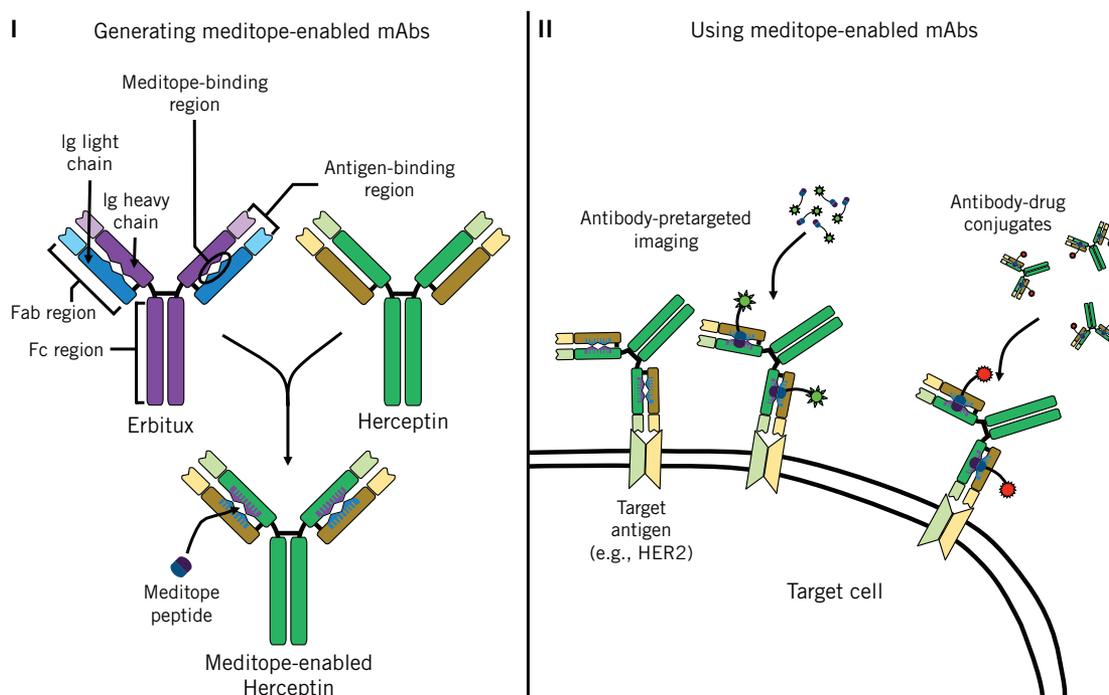


Figure 1. Mediotope-enabled mAbs. As reported by Donaldson *et al.*, the Fab framework of the chimeric anti-epidermal growth factor receptor (EGFR) mAb Erbitux cetuximab contains a unique peptide-binding site that is distinct from the antibody's antigen-binding sites. This site is located in the center of a cavity formed by the mAb's light and heavy chains and can bind to engineered peptides called mediotopes without interfering with antigen binding.

[I] The mediotope-binding site can be engineered into other mAbs, such as the anti-HER2 (EGFR2; ErbB2; neu) mAb Herceptin trastuzumab.

Mediotope-enabled mAbs can be conjugated to a broad range of molecules and thus represent a new avenue in antibody-drug conjugates. A mediotope-enabled mAb can be used to target many otherwise nonspecific agents without the need to make additional modifications to the mAb itself.

[II] Potential applications include using mediotope-enabled mAbs plus mediotope-peptide conjugates to deliver imaging agents [green stars] or therapeutic payloads [red stars] to target cells.

compound. Instead, all the chemistry and conjugation work now occurs on the mediotope peptide, which Williams said is easier to work with than a mAb.

The other key benefit of mediotope-enabled mAbs is that users can swap out one mediotope conjugate for another. This enables the generation and evaluation of a series of candidates more efficiently than methods that involve chemical modifications to the mAb itself.

"What we've basically done is installed a hitch onto an antibody that would allow it to carry a variety of payloads," Williams told *SciBX*. "We could build libraries with hundreds of mediotopes conjugated with different drugs and imaging agents, all of which could be attached to a single mediotope-enabled mAb without making additional modifications to the mAb itself."

He also said that building a mediotope-binding site into a mAb should not increase its immunogenicity. The reason is that the location of the binding site and the specific amino acid sequences that mediate mediotope binding are unlikely to be seen or recognized by the host immune system.

In contrast, Williams said that reengineered mAbs often result in the

exposure of new antibody surfaces to the host immune system, which can be immunogenic.

Grewal said that the mediotope platform also enables the design of ADCs with specific and predictable drug-to-antibody ratios, which could allow for better control over efficacy and toxicity compared with current ADC linker technologies.

He also said that a mediotope could noncovalently bind to a mAb under a broad range of settings, such as aqueous, nonaqueous, hydrophobic, acidic or basic conditions.

"This provides for a very easy process in which drug-loaded mediotopes and antibodies can simply be mixed to create fully functional antibody-drug conjugates," he said.

Making more mAbs

Williams said that the group at City of Hope plans to report additional examples of mediotope-enabled mAbs in an upcoming publication. He said that the group also is evaluating various strategies to improve the affinity of mediotope-mAb interactions.

Mediotope Biosciences president and CEO Stephanie Hsieh said that

“In order to validate the clinical utility of the meditope technology, it must be studied through an *in vivo* evaluation using well-established model systems.”

—*Iqbal Grewal, ImmunGene Inc.*

the meditope platform tested *in vivo*.

“In order to validate the clinical utility of the meditope technology, it must be studied through an *in vivo* evaluation using well-established model systems,” he told *SciBX*. “In addition, toxicity studies in animals are necessary to validate its viability for potential therapeutic use.”

City of Hope has filed patents covering the meditope platform, which have been exclusively licensed to Meditope Biosciences.

the company will focus on developing the meditope platform for clinical applications. She said that the most immediate application will be using the platform as a new way to generate ADCs.

Grewal said that he now wants to see candidates generated with

Hsieh said that Meditope is seeking deals to generate meditope-enabled versions of a partner's mAbs.

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KCC2 escape from neuropathic pain

By Benjamin Boettner, Assistant Editor

A Canadian team has published preclinical proof of concept that activating a transporter called KCC2 represents a new mechanism for treating neuropathic pain.¹ The group expects that compounds against the ion transporter will be safer than marketed neuropathic pain drugs because its effects are restricted to malfunctioning neurons.

The next step is developing more drug-like molecules against KCC2 (solute carrier family 12 potassium-chloride transporter member 5; SLC12A5) as the first iteration of activators had bioavailability issues.

Neuropathic pain is caused by insults to the nervous system that disrupt the balance between excitatory, pain-causing stimuli and inhibitory, pain-suppressing stimuli. This imbalance can persist long after the initial insult.²

The condition, also called hyperalgesia, is treated with a variety of compounds against a range of targets. These include **Pfizer Inc.**'s Lyrica pregabalin, a GABA receptor agonist; **Teva Pharmaceutical Industries Ltd.**'s Effentora, a μ -opioid receptor (OPRM1; MOR) agonist; **NeurogesX Inc.**'s Qutenza capsaicin, a transient receptor potential vanilloid 1 (TRPV1; VR1) agonist; **Elan Corp. plc**'s Prialt ziconotide, an N-type Ca^{2+} channel blocker; and **Eli Lilly and Co.**'s selective serotonin and norepinephrine reuptake inhibitor, Aricclaim duloxetine.

Most of the drugs have limited efficacy and can elicit side effects on neuronal functions including impaired motor abilities, numbing and physical dependency.

KCC2 maintains the low intracellular Cl^- levels needed for normal neuronal function by constantly pushing Cl^- ions out of the cell. KCC2 impairment causes the Cl^- gradient to collapse.

Previous work has shown that inhibitory stimuli in GABAergic neurons can be lost when KCC2 activity is compromised and that this can lead to neuropathic pain.³ In rats, microglial cells induce Kcc2 activity as a mechanism to counter Cl^- imbalances caused by nerve injury.⁴

Now, a team led by **Laval University**'s Yves De Koninck has identified a compound that stimulates KCC2 activity and shown that it relieved pain in rats.

De Koninck is a professor of psychiatry and neuroscience at Laval and scientific director at the **Hospital Center of Quebec Research Center**.

Using a fluorescent Cl^- extrusion assay, the researchers identified and optimized a compound, CLP257, that had nanomolar activity and was highly selective for KCC2 over related Cl^- transporters.

In spinal slices taken from rats with neuropathic pain, CLP257 enhanced Kcc2 activity and normalized measures of pain sensation, including electrophysiological nerve responses and mechanical

withdrawal behavior.

Next, the team developed a prodrug called CLP290 that provided higher serum levels of CLP257 than the original compound. In rats, the analgesic effect of CLP290 was similar to that of Lyrica. Unlike Lyrica, the prodrug did not numb motor functions.

Findings were reported in *Nature Medicine*.

"KCC2 represents a fresh avenue to pain medication because stimulating KCC2 normalizes endogenous pain inhibition," said De Koninck. "In normal neurons, Cl^- levels are kept very low. Therefore, the effect of KCC2 enhancers will mainly touch on troubled neurons with elevated Cl^- levels."

"The strategy could have significant advantages over other therapeutic agents like opioids, Lyrica pregabalin and calcium channel blockers, which mostly regulate ion channels and not active transporters," added De Koninck.

"The mantra is that the best drugs for neuropathic pain—the gabapentinoids—are only effective in about 30% of patients, and those patients on average only get about 30% relief. So clearly new drugs are essential," said Allan Basbaum, chair of the Department of Anatomy at the **University of California, San Francisco**.

"The mantra is that the best drugs for neuropathic pain—the gabapentinoids—are only effective in about 30% of patients, and those patients on average only get about 30% relief. So clearly new drugs are essential."

—Allan Basbaum,
University of California,
San Francisco

Targeting KCC2

Based on earlier KCC2 findings, De Koninck cofounded Chlorion Pharma Inc. to develop KCC2 agonists for neuropathic pain.

The company raised C\$6 million (\$6.1 million) in 2007, but Chlorion's first series of activators ran into bioavailability issues in a preclinical study in dogs. The molecules did show high KCC2 specificity and no on-target toxicity.

The company set about fixing the bioavailability issue and identified an undisclosed series of KCC2 agonists but ultimately ran out of money and closed its doors in 2011. Rights to the activators reverted back to Laval University.

De Koninck said that his team since has shown markedly improved bioavailability for the new agonists and is in talks with industry.

Regardless of bioavailability, Michael Gold, a professor of anesthesiology at the **University of Pittsburgh**, said that it will be important to examine KCC2 stimulation in assays that better reflect ongoing pain such as the conditioned place preference assay.

Ronald Burch, CMO of **Naurex Inc.**, said that it will also be important to investigate KCC2 stimulation in multiple neuropathic pain models. The reason, he said, is that the condition can have many causes, including physical nerve injury and metabolic injury in diabetic neuropathy, as well as inflammatory and infectious diseases like postherpetic neuralgia.

Gold agreed, noting that inflammatory pain models in particular do not rely on spontaneously incurred physical injury. Although the models in the paper used pain that sets in after an initial physical injury subsides, inflammatory pain models likely involve different pain mechanisms altogether and would help show that KCC2 stimulation works more broadly.

Naurex has completed a Phase I trial of GLYX-13, an NMDAR

“KCC2 represents a fresh avenue to pain medication because stimulating KCC2 normalizes endogenous pain inhibition.”

— *Yves De Koninck, Laval University*

modulator, to treat neuropathic pain.

Ruth McKernan, CSO of Pfizer's Neusentis unit, told *SciBX* that “it will be important to quantitatively assess target engagement at relevant sites of action *in vivo* to aid in predicting human exposure required for both efficacy and side

effects. This would be a high priority for the target class since we really do not know what is required in man and how preclinical results will translate to clinical studies.”

Basbaum and Gold also wanted to see more detailed dose-response curves for KCC2 stimulation in assays of neuropathic pain and motor functions. Such data would help define the therapeutic window of KCC2 stimulation.

Finally, Gold noted that it will be necessary to determine “how long the drug effects last and whether the drug can be repeatedly administered and still take effect. Time-course studies can show whether the drug-induced Cl⁻ shift persists or whether the cells compensate for it long term.”

Basbaum added, “As the best drugs for neuropathic pain are generally also anticonvulsants, it is of interest to learn whether the new compound acts in a fashion similar, at least clinically. If not, then one can conclude that clearly it is working via a very different mechanism. That also argues that some combination of the gabapentinoids and the new compound could prove especially valuable.”

Wider horizons

Besides neuropathic pain, other neurological disorders with imbalances in Cl⁻ homeostasis, like epilepsy, migraine or anxiety, could benefit from KCC2 stimulation.

De Koninck plans to study KCC2-targeted compounds in an epilepsy model in which epilepsy-related neurological changes develop before they eventually trigger epileptic episodes. The model will provide insights on the effects of KCC2 agonists on seizure-evoking hyperexcited neurons and on changes in neuronal networks.

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Benztropine for MS

By Lauren Martz, Staff Writer

Although the secondary progressive multiple sclerosis space is filling up with new compounds, few have shown the ability to remyelinate neurons. A new study suggests that an ancient Parkinson's disease drug—benztropine—could accomplish that task in secondary progressive multiple sclerosis, although it might require reformulation to improve activity and specificity.¹

There are multiple immunomodulatory therapies on the market that stop or slow the autoimmune attack on neurons by myelin-specific T cells in relapsing-remitting MS (RRMS). Nevertheless, within 20 years of diagnosis, the vast majority of patients will progress to secondary progressive MS (SPMS). At that point, available immunomodulatory agents are no longer able to prevent further neuronal degeneration.

The only drug on the market for SPMS is mitoxantrone, a generic chemotherapeutic that carries a boxed warning about potential cardiotoxicity.

The SPMS pipeline includes seven compounds in clinical development. A few compounds, including the antibodies BIIB033 and HIGM22, potentially can remyelinate axons and thus reverse the course of the disease.

BIIB033, from **Biogen Idec Inc.**, targets leucine-rich repeat neuronal protein 1 (LINGO-1) and is in Phase II testing. HIGM22, a remyelinating antibody from **Acorda Therapeutics Inc.**, is in Phase I testing.

Now, a group of researchers from **The Scripps Research Institute** and the **California Institute for Biomedical Research** (Calibr) have developed a screen for small molecules that can accomplish remyelination.

Specifically, the screen identifies compounds that promote the differentiation of oligodendrocyte precursor cells (OPCs) into myelin-promoting mature oligodendrocytes. In MS, the differentiation process is impaired.^{2,3}

The team was led by Peter Schultz, a professor of chemistry at Scripps and founding director of Calibr; Luke Lairson, an assistant professor at Scripps and a principal investigator at Calibr; and Brian Lawson, an assistant professor of immunology at Scripps. The team also included researchers from the **Salk Institute for Biological Sciences** and **Hokkaido University**.

The group screened about 100,000 small molecules in rat OPCs using an imaging assay that detects expression of myelin basic protein (MBP). MBP expression indicates that the OPCs have differentiated into mature oligodendrocytes.

From the screen's hits, the team selected benztropine for additional studies because it is approved for PD, is orally bioavailable and crosses the blood brain barrier.

In coculture of mouse or rat OPCs with mouse neurons, benztropine induced OPC differentiation and axon remyelination by increasing the number of myelinating oligodendrocytes.

Benzotropine functions through multiple mechanisms including acting as an anticholinergic and antihistamine and inhibiting dopamine

reuptake. The group blocked each mechanism to determine which one was responsible for OPC differentiation.

They found that a muscarinic acetylcholine receptor agonist, but not nicotinic acetylcholine, histamine or dopamine agonists, blocked the OPC-differentiating effect of benztropine.

Muscarinic receptor antagonists induced OPC differentiation in cultures, thus suggesting that the muscarinic antagonist effect of benztropine, specifically M1 and M3 antagonism, is responsible for OPC differentiation.

In a mouse model of experimental autoimmune encephalomyelitis (EAE), intraperitoneal injection of 10 mg/kg a day of benztropine decreased clinical severity compared with vehicle control injection and prevented the disease relapse phase. Benztropine also decreased disease severity comparably to or better than the marketed MS immunosuppressants Avonex IFN- β -1a and Gilenya fingolimod (FTY720).

Biogen Idec markets Avonex IFN- β -1a and **Bayer AG** markets Betaseron IFN- β -1b to treat RRMS. **Novartis AG** and **Mitsubishi Tanabe Pharma Corp.** market Gilenya, a sphingosine 1-phosphate receptor antagonist, to treat RRMS.

In spinal cords from EAE mice that received prophylactic benztropine, immune cell infiltration and demyelination were the same as those in vehicle-treated mice.

However, the number of mature oligodendrocytes increased in the mice given benztropine. These findings suggest that benztropine induced remyelination without altering the immune response.

In a mouse model of cuprizone-induced, T cell-independent demyelination, 10 mg/kg per day of benztropine accelerated remyelination following cuprizone withdrawal, whereas vehicle had no effect.

Finally, the team tested the effects of benztropine in combination with immunosuppressive MS therapeutics. In EAE mice, 2.5 mg/kg of benztropine plus 1 mg/kg of Gilenya was better than Gilenya alone in multiple measures of disease and comparable to Gilenya monotherapy in others.

Also, 2.5 mg/kg of benztropine plus a 0.1 mg/kg dose of Gilenya produced decreases in disease severity that were comparable to 1 mg/kg of Gilenya alone.

The results were published in *Nature*.

Complementary remyelination

Lairson told *SciBX* that his team is working on pharmacokinetic tissue-distribution studies. "Upon completion of these studies we plan to meet with a clinical consultant to determine our best course," said Lairson.

He said that could include studies of benztropine in MS either alone or in combination with immunosuppressive therapy.

"Benztropine seems to have little or no impact on the immune system but rather seems to drive differentiation of the precursors of myelin cells to actual myelinating cells, stimulating myelin production," said Mike Gresser, CSO of the **Myelin Repair Foundation**.

"This approach represents a much-needed addition to MS therapeutics," added Spyros Deftereos, SVP of drug discovery at **Biovista**

"This approach represents a much-needed addition to MS therapeutics. We need immune modulators, neuroprotectants and remyelinating agents to all work together."

— *Spyros Deftereos, Biovista Inc.*

Inc. “It is important to treat the neurological deficits when patients relapse, and in addition to dampening the neuron-damaging immune response in these patients, we need a way of restoring the myelin to repair the damage. We need immune modulators, neuroprotectants and remyelinating agents to all work together.”

Biovista’s BVA101 and BVA201 are in preclinical testing to treat MS. The mechanisms are undisclosed.

“The goal is not just to stabilize patients by blocking further damage but also to positively improve their disability,” said Don Healey, CSO of **Opexa Therapeutics Inc.**

Opexa’s Tcelna, a patient-specific T cell immunotherapy that decreases myelin-reactive T cells, is in Phase IIb testing to treat SPMS.

Andrew Blight, CSO of Acorda, said that it may be worthwhile to try combinations of remyelinating agents.

In addition to developing HIGM22, Acorda markets Ampyra dalfampridine, a sustained-release formulation of 4-aminopyridine (4-AP), to improve walking and other neurological conditions in patients with MS.

Beyond benzotropine

There are two main roadblocks for benzotropine—one is molecule specific, and the other is indication specific.

For the disease in general, results in EAE models do not consistently predict results in patients with MS.

“One reason that EAE is not predictive of MS is that animal models are conducted over a short time frame of typically a month or two. By comparison, MS is truly a chronic disease taking many years to evolve to a progressive stage that exhibits changes from acute attacks that translate to relapses through to chronic progression, which may be driven by different disease processes. Relapses can be characterized by cellular proinflammatory events, whereas progression bears the hallmarks of chronic inflammation, possibly involving innate immune mechanisms,” said Healey.

He added that the big problem in the field is coming up with a model for SPMS, which goes beyond the typical proinflammatory events that characterize chronic relapsing EAE. No models to date truly capture the immunopathology of progressive MS, he said.

Deftereos told *SciBX* that “the mouse data show that benzotropine is more potent than existing drugs such as FTY720 and IFN- β , which is good, but it also shows that the existing drugs are equally effective in mice. In humans with MS, FTY720 is much more potent than IFN- β , so it isn’t yet clear how reliable the data are or how effective benzotropine may be in humans.”

Despite the problems with the EAE model, Lawrence Steinman, a professor of neurology and of neurological sciences and pediatrics at **Stanford University**, added that effective and approved drugs for MS, including Tysabri natalizumab and Gilenya, have all come out of the EAE model. Natalizumab is marketed by **Elan Corp. plc** to treat MS.

“EAE, when used creatively, can give some potential drug candidates that will make a difference in MS treatment,” he added.

Lairson countered that the team also showed strong results in the cuprizone model, although he acknowledged that “it hasn’t really been evaluated whether results in this model will translate. There are not any effective remyelinating agents in humans at this point to use as indicators.”

Healey said that the downside of the cuprizone model is that although it enables a state of demyelination to be generated, which is reversible and leads to spontaneous remyelination that was accelerated by benzotropine, it is achieved in the absence of an opposing chronic autoimmune response that typifies progressive MS.

Regardless of the model, Deftereos said that the data shown suggest that benzotropine is effective at much higher doses than those approved for human use and causes neurological symptoms like confusion at high doses.

Healey added that the dose-limiting neurological adverse events represent a significant challenge, but the possibility of defining a new chemical entity targeting the associated signaling pathway in oligodendrocytes with lower toxicity would be an enviable goal.

Blight added, “The dosing used in preclinical models was high relative to the maximum tolerable dose in humans, and the drug was delivered by intraperitoneal injection rather than as an oral medicine.”

“It will be important to investigate whether the concentrations in the brains of patients taking approved doses of benzotropine are parallel to the efficacious tissue drug levels achieved in the brains of EAE models and *in vitro* assays,” said Gresser.

Lairson said that medicinal chemistry could improve the therapeutic index of benzotropine.

Gresser said that a path forward could involve “identifying the molecular targets of the promyelinating effects of benzotropine and then finding a compound that has the desired effect on this target at efficacious doses free of the adverse effects of benzotropine.”

Lairson said that in addition to benzotropine, his team has identified six other clinical candidates using the screen that could become remyelinating therapeutics.

Scripps has filed a patent application covering the use of a broad panel of neurotransmitter receptor–modulating agents in MS, including benzotropine. The IP is available for licensing.

Martz, L. *SciBX* 6(42); doi:10.1038/scibx.2013.1180
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COMPANIES AND INSTITUTIONS MENTIONED

Acorda Therapeutics Inc. (NASDAQ:ACOR), Ardsley, N.Y.
Bayer AG (Xetra:BAYN), Leverkusen, Germany
Biogen Idec Inc. (NASDAQ:BIIB), Weston, Mass.
Biovista Inc., Charlottesville, Va.
California Institute for Biomedical Research, La Jolla, Calif.
Elan Corp. plc (NYSE:ELN), Dublin, Ireland
Hokkaido University, Sapporo, Japan
Mitsubishi Tanabe Pharma Corp. (Tokyo:4508; Osaka:4508), Osaka, Japan
Myelin Repair Foundation, Saratoga, Calif.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Opexa Therapeutics Inc. (NASDAQ:OPXA), The Woodlands, Texas
Salk Institute for Biological Sciences, La Jolla, Calif.
The Scripps Research Institute, La Jolla, Calif.
Stanford University, Stanford, Calif.

Personal factors

By Chris Cain, Senior Writer

A multi-institute team collaborating with the FDA has determined why patients with a form of severe hemophilia rarely develop neutralizing antibodies against factor VIII replacement therapies. The team is also developing an algorithm to predict the likelihood of antibody development against other recombinant protein-based therapies.¹ Based on the results, one of the investigators has cofounded **Haplomics Inc.** to develop new factor VIII replacement therapies.

Hemophilia A is caused by inherited alterations in the *F8* gene that encodes factor VIII, a critical component of the coagulation cascade. Standard of care for the disease is treatment with plasma-derived or recombinant factor VIII protein.

At least eight companies market factor VIII replacement products.

Because these products are given to patients whose normal factor VIII is mutated, the immune system can deem the replacement protein a foreign antigen and produce neutralizing antibodies against it.

These neutralizing antibodies, commonly known as inhibitors, develop less frequently in patients with *F8* missense mutations, who have mild to moderate forms of the disease. The inhibitors develop more frequently in patients with large deletions in *F8*, who have severe forms of the disease, because their immune systems are not tolerized to the normal form of the protein.

A long-standing question has been why development of inhibitors is infrequent in a subset of patients with severe disease—those carrying an inversion in intron 22 (I22) of *F8*. Like patients with *F8* deletions, these patients lack antigenically cross-reactive material in their plasma. But only about 20% of patients with the inversion develop inhibitors, whereas up to 88% of patients with deletions develop inhibitors.

To investigate this discrepancy, a team led by Tom Howard, director of the Hemostasis Laboratory and co-director of the Hemostasis and Transfusion Medicine Consult Service at the **VA Greater Los Angeles Healthcare System**, and Zuben Sauna, a visiting scientist at the Division of Hematology in the FDA's Center for Biologics Evaluation and Research, set out to characterize *F8* and factor VIII in these patients in greater detail.

Howard is also director of the pharmacogenetics section of the VA's Molecular Pathology Laboratory and an associate professor of pathology and laboratory medicine at the **Keck School of Medicine of the University of Southern California**.

RT-PCR and protein expression analysis of cell lines derived from a patient with the I22 inversion showed that the entire protein was produced within cells as 2 separate polypeptides, 1 comprising the first 2,143 amino acids of the protein and 1 comprising the last 16 amino acids.

This meant that although factor VIII was not functional in these patients, the full-length sequence of the protein was still being produced. The researchers used immunohistochemistry to confirm the expression of these factor VIII polypeptides in numerous cell types and showed that the lack of factor VIII function was likely because the peptides could not be secreted by cells.

RT-PCR of blood samples from 25 additional patients found that both factor VIII polypeptides were produced in all cases.

Valder Arruda, an associate professor of pediatrics at the **Perelman School of Medicine at the University of Pennsylvania** and **The Children's Hospital of Philadelphia**, told *SciBX* that these results clarified a long-standing question in the hemophilia community.

"This was a puzzle for the field until this paper came out. We have called patients cross-reactive material [CRM] positive or CRM negative based on an analysis of plasma from patients. What this group is saying is that in people with this I22 inversion, they are CRM negative in the plasma but CRM positive intracellularly, and their immune system does see pieces of factor VIII," he said.

Having established a rational explanation for why these patients often do not develop inhibitors, the research team next set out to better understand why some I22 inversion carriers develop inhibitors and others do not.

First, the group sequenced *F8* in the patients with I22 to identify regions of potential mismatch between each patient and factor VIII replacement therapy. They expected that some of this mismatch would be due to differences between the replacement protein and the junction region that normally bridges the two

halves of the separate factor VIII polypeptides made in these patients.

An additional source of mismatch would be nonsynonymous polymorphisms within the *F8* gene found in different ethnic populations, which carry slightly different haplotypes of the gene.

The team identified all overlapping 15-mer peptides that include these mismatched positions—this is the size of peptides that are presented by major histocompatibility complex class II (MHCII) proteins. This information was combined with genotyping of each individual's *MHCII DR β1 (HLA-DRB1)* locus to predict foreign peptides with high affinity for *HLA-DRB1* that might induce an immune response.

The group found that, as expected, the number of high-affinity foreign peptide-HLA-DRB1 complexes could significantly predict inhibitor development and was more effective than simply counting the number of predicted foreign peptides alone. This suggests that polymorphisms within the genes could indeed affect the risk of inhibitor development.

Results were published in *Nature Medicine*. Howard was previously a visiting professor of hematology and oncology in the Department of Medicine at the **University of California, Los Angeles David Geffen School of Medicine**.

Niche populations

Howard and Sauna said that the findings could help explain why African-American patients are more likely to develop inhibitors than other ethnic groups.

In a 2009 study published in *The New England Journal of Medicine*, Howard led a team that showed African Americans more frequently carry an *F8* haplotype that is different from the haplotype used to generate recombinant factor VIII replacement products.² Moreover, the frequency of inhibitor development was higher in patients with those haplotypes.

In the *Nature Medicine* paper, an analysis of an independent cohort of 313 patients with known race, I22 status and inhibitor status showed a statistically significant increase in inhibitor development in African-

"We want to develop a predictive, diagnostic tool that would facilitate the treater's ability to make more informed choices about factor VIII replacement products."

— Vincent LaTerza, Haplomics Inc.

American patients with the I22 inversion compared with European-American patients with I22.

Howard told *SciBX*, “These nonsynonymous *F8* polymorphisms, which occur much more frequently in African-American patients, represent additional determinants of immunogenicity risk that may explain why the prevalence of antidrug antibodies in this population is twice that observed in European Americans.”

In 2004, Howard cofounded Haplomics based on early findings related to this work.

“What distinguishes our approach is that we are examining what factor VIII protein material the patient is making internally before making a decision as to which product is optimal for the patient,” Haplomics cofounder and CEO Vincent LaTerza told *SciBX*. “We want to develop a predictive, diagnostic tool that would facilitate the treater’s ability to make more informed choices about factor VIII replacement products. Ideally, factor VIII products that closely match what factor VIII the patient expresses—for example, their haplotype—could be administered to as many patients as possible.”

LaTerza acknowledged that the market is a subset of an already small patient population but said that the business case can be made given the high cost required to treat patients with hemophilia who develop anti-factor VIII antibodies. “In our view, failing to take into account what factor VIII material a patient will likely tolerate is a major missed opportunity for treaters and, more importantly, for hemophilia A patients at risk for inhibitors,” he said.

Glenn Pierce, SVP of global medical affairs and CMO of **Biogen Idec Inc.**’s hemophilia therapeutic area, told *SciBX* that he wants to see more clinical data to back up the utility of the predictive algorithm.

“The numbers of people with I22 inversion and the specific haplotype variants discussed in this paper are very small. In order to test the hypotheses generated, it would be important to conduct a clinical trial just in these patients and particularly in a subset of them who had never been treated with a factor VIII therapy, if feasible. Until that kind of work can be done, it would be difficult to know if the algorithm developed could have significant clinical utility. However, the algorithm shows initial promise and indicates a variety of directions for further research,” he said.

Arruda agreed. “While there is an association between haplotype and risk of inhibitor development, it has not been proven that it is due to the haplotype. To demonstrate that, you have to show that patients with inhibitors have T cell epitopes with haplotype-specific amino acids,” he said.

Pierce was a coauthor on the *Nature Medicine* study. Biogen’s long-acting Eloctate rFVIII-Fc has completed Phase III testing and is under FDA review. The company is also collaborating with multiple not-for-profit organizations including the **National Hemophilia Foundation** to provide financial and scientific support for genotyping patients with hemophilia A and B.

Howard and Sauna both agreed that the key next step for their pharmacogenomic analysis is to examine whether the peptide predictions hold up in a clinical trial.

“We hope to establish clinical collaborations to identify actual peptides presented by major histocompatibility complex proteins of hemophilia A patients with the I22 inversion,” said Sauna. “If the peptide sequences are those that would be predicted based on the current work, this will support our hypothesis. This, in turn, may lead to development of new strategies for products to minimize the risk from immunological reactions for at-risk subpopulations or ethnicities.”

Broad applications

Sauna thinks that the team’s approach has applications outside of hemophilia A. “Numerous bioengineered proteins are entering the drug development pipeline. Such engineered proteins always have neopeptides that are not found in nature. These can sometimes trigger immune responses. We are currently applying our methods to

understand whether neopeptides generated during the design of clotting factors may cause them to be immunogenic,” he said.

“If we can validate our approaches, at-risk individuals could potentially be identified in early stages of drug development, opening the door to clinical trials based on pharmacogenetic characteristics,” he added. “This paradigm would ultimately facilitate the development of drugs by identifying and excluding the few individuals for whom the drug is unsuitable.”

Søren Erik Bjørn, VP of hemophilia R&D at **Novo Nordisk A/S**, agreed that the algorithm is promising, contingent on further clinical validation.

“In principle, this approach could be generally applicable, not only to benefit the hemophilia population but also in other areas where you have peptide drugs prone to an immunogenic response. Clinical studies within hemophilia will be challenging due to the small number of patients available, and it needs to be validated in larger cohorts, like rheumatoid arthritis,” he said.

He said that the algorithm is particularly interesting for its incorporation of information about the HLA genotype of patients and said that Novo Nordisk uses similar types of computational and *in vitro* analyses to assess the potential immunogenicity of the recombinant protein products it develops.

Novo Nordisk markets NovoSeven, a recombinant human coagulation factor VIIa, to treat patients with factor VII deficiency and those who have acquired hemophilia or factor VIII or factor IX deficiency and who have developed inhibitors. In October, the FDA approved Novoeight turoctocog alfa recombinant factor VIII to treat hemophilia A patients with factor VIII deficiency.

Pierce and Bjørn both sounded notes of caution on whether there is sufficient evidence to engage in the development of personalized therapies for hemophilia A.

“Currently, we know that certain gene mutations carry increased risk of inhibitors, but we cannot predict with a high degree of accuracy who will develop an inhibitor and who will not. It is important to understand with more certainty who is likely to form an inhibitor, as well as develop new therapeutic approaches, before envisioning a time

“We hope to establish clinical collaborations to identify actual peptides presented by major histocompatibility complex proteins of hemophilia A patients with the I22 inversion.”

—Zuben Sauna,
Food and Drug Administration

when clinicians could optimally guide treatment selections for their patients,” said Pierce.

He added, “For this small subset of the hemophilia population, the contribution of haplotype risks remains unclear; it may be one of a number of factors that could potentially affect inhibitor risk. Larger NIH-sponsored clinical studies are ongoing to assess haplotype mismatch between patients and replacement therapies to determine if there is a higher risk for inhibitor development. Developing haplotype-specific treatments would be challenging given the current significant regulatory steps required for the development of all hemophilia therapies.”

According to Bjørn, “There might be a need for several factor VIII molecules to significantly reduce inhibitor formation, and such development would be a huge task for any company. We should also look into other technologies and single products that more broadly prevent inhibitor development in patients with hemophilia.”

VA Los Angeles has filed for patents covering the algorithm, and the IP is licensed to HaploMics.

Cain, C. *SciBX* 6(42); doi:10.1038/scibx.2013.1181
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COMPANIES AND INSTITUTIONS MENTIONED

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Food and Drug Administration, Rockville, Md.
HaploMics Inc., Atlanta, Ga.
Keck School of Medicine of the University of Southern California, Los Angeles, Calif.
National Hemophilia Foundation, New York, N.Y.
Novo Nordisk A/S (CSE:NVO; NYSE:NVO), Bagsvaerd, Denmark
Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pa.
University of California, Los Angeles David Geffen School of Medicine, Los Angeles, Calif.
VA Greater Los Angeles Healthcare System, Los Angeles, Calif.

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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				
Brain cancer	Colony-stimulating factor 1 receptor (CSF1R; C-FMS; CD115)	<p>Mouse studies suggest the brain-penetrant CSF1R inhibitor BLZ945 could be used to treat glioblastoma multiforme (GBM). In a mouse model of platelet derived growth factor B (PDGFB; PDGF2)-driven proneural GBM, BLZ945 inhibited tumor-associated macrophages and blocked glioma growth, and it increased survival compared with vehicle. In mice with proneural GBM xenografts, BLZ945 decreased tumor growth, tumor invasion and expression of activated macrophage markers associated with tumor-promoting functions compared with vehicle. Next steps could include using CSF1R inhibitors in combination with glioma cell-directed therapies. The Novartis Institutes for BioMedical Research synthesized BLZ945 and collaborated on this study. The development status of the compound is undisclosed.</p> <p>Array BioPharma Inc. has the small molecule CSF1R inhibitor ARRY-382 in Phase I testing to treat cancer.</p> <p>Amgen Inc. has the humanized mAb AMG 820 in Phase I trials to treat cancer.</p> <p>Eli Lilly and Co. has the humanized mAb IMC-CS4 in Phase I testing to treat solid tumors.</p> <p>Roche has the humanized mAb RG7155 in Phase I trials to treat solid tumors.</p> <p>SciBX 6(42); doi:10.1038/scibx.2013.1182 Published online Oct. 31, 2013</p>	Patent application filed by Novartis AG and the Memorial Sloan-Kettering Cancer Center; licensing status undisclosed	<p>Pyonteck, S.M. <i>et al. Nat. Med.</i>; published online Sept. 22, 2013; doi:10.1038/nm.3337</p> <p>Contact: Johanna A. Joyce, Memorial Sloan-Kettering Cancer Center, New York, N.Y.</p> <p>e-mail: joyce@mskcc.org</p>
Cancer	CD3; folate receptor	<p><i>In vitro</i> and mouse studies suggest an anti-CD3 antibody conjugated to folic acid could help treat cancer. The antibody conjugate specifically bound folate receptor-expressing tumor cells and was cytotoxic when cultured with peripheral blood mononuclear cells (PBMCs). In mice with folate receptor-positive nasopharyngeal cancer xenografts, the conjugate plus human PBMCs decreased tumor volume compared with saline plus PBMCs. Next steps include safety studies. This work was performed in collaboration with Ambrx Inc., and the development status of the antibody was not disclosed.</p> <p>SciBX 6(42); doi:10.1038/scibx.2013.1183 Published online Oct. 31, 2013</p>	Patent and licensing status unavailable	<p>Kularatne, S.A. <i>et al. Angew. Chem. Int. Ed.</i>; published online Sept. 25, 2013; doi:10.1002/anie.201306866</p> <p>Contact: Peter G. Schultz, The Scripps Research Institute, La Jolla, Calif.</p> <p>e-mail: schultz@scripps.edu</p>
Cancer	Ribonucleotide reductase M2 (RRM2; R2)	<p>Cell culture and mouse studies identified RRM2 inhibitors that could help treat drug-resistant cancers. In cancer cell lines resistant to the ribonucleotide reductase inhibitors hydroxyurea and Gemzar gemcitabine, the lead RRM2 inhibitor decreased proliferation compared with hydroxyurea or gemcitabine alone. In mouse xenograft models of human ovarian cancer or leukemia, the inhibitor caused tumor regression. Next steps could include testing the inhibitor in additional cancer models.</p> <p>Eli Lilly and Co. markets Gemzar to treat breast, ovarian, pancreatic and prostate cancers.</p> <p>Sanofi markets the ribonucleotide reductase inhibitor Clolar clofarabine to treat acute lymphoblastic leukemia (ALL).</p> <p>Calando Pharmaceuticals Inc.'s CALAA-01, a small interfering RNA duplex targeting the M2 subunit of RRM2 and delivered via the RNAi/oligonucleotide nanoparticle delivery (RONDEL) system, is in Phase I testing to treat solid tumors.</p> <p>SciBX 6(42); doi:10.1038/scibx.2013.1184 Published online Oct. 31, 2013</p>	Patent and licensing status unavailable	<p>Zhou, B. <i>et al. Cancer Res.</i>; published online Sept. 26, 2013; doi:10.1158/0008-5472.CAN-13-1094</p> <p>Contact: Yun Yen, City of Hope, Duarte, Calif.</p> <p>e-mail: yuyen@coh.org</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer	Tubulin	<i>In vitro</i> and mouse studies suggest azaindole-based tubulin inhibitors could help treat cancer. Chemical synthesis, SAR studies and <i>in vitro</i> testing of azaindole analogs identified a lead compound that bound tubulin to inhibit its polymerization at single-digit nanomolar IC ₅₀ values. The lead compound also inhibited proliferation of human cervical, colorectal, stomach and lung cancer cell lines at low nanomolar IC ₅₀ values. In mice bearing human colon tumors, the lead compound decreased tumor growth compared with vehicle. Future studies could include testing the lead compound in additional xenograft tumor models. SciBX 6(42); doi:10.1038/scibx.2013.1185 Published online Oct. 31, 2013	Patent and licensing status unavailable	Lee, H.-Y. <i>et al. J. Med. Chem.</i> ; published online Sept. 24, 2013; doi:10.1021/jm4011115 Contact: Jing-Ping Liou, Taipei Medical University, Taipei, Taiwan e-mail: jpl@tmu.edu.tw Contact: Jang-Yang Chang, National Health Research Institutes, Tainan, Taiwan e-mail: jychang@nhri.org.tw
Lung cancer	Carbonic anhydrase XII (CAXII)	Cell culture and mouse studies identified an anti-CAXII mAb that could help treat cancer. In membrane fractions isolated from a lung cancer cell line, the mAb inhibited CAXII activity with an IC ₅₀ of about 1 µg/mL. In two tumor cell lines, the anti-CAXII mAb decreased cell proliferation compared with a control antibody. In mouse xenograft models of lung cancer, the anti-CAXII mAb decreased tumor growth compared with control antibody. Next steps include humanizing the mAb and testing it in additional cancer types. SciBX 6(42); doi:10.1038/scibx.2013.1186 Published online Oct. 31, 2013	Patent application filed; available for licensing	Gondi, G. <i>et al. Cancer Res.</i> ; published online Sept. 12, 2013; doi:10.1158/0008-5472.CAN-13-1110 Contact: Reinhard Zeidler, Ludwig Maximilians University of Munich, Munich, Germany e-mail: reinhard.zeidler@med.lmu.de
Prostate cancer	Second chromosome locus associated with prostate-1 (SchLAP1; LINC00913)	Cell culture and mouse studies suggest inhibiting the long noncoding RNA (lncRNA) SchLAP1 could help treat prostate cancer. An analysis of gene expression data from prostate cancer samples showed an association between high SchLAP1 expression and increased risk of disease recurrence and clinical progression. In cultured prostate cancer cells and a mouse xenograft model of prostate cancer, SchLAP1 knockdown decreased invasiveness and tumor metastasis compared with knockdown of a control gene. Cell culture and biochemical assays showed that SchLAP1 interacted with and antagonized tumor-suppressive SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily b member 1 (SMARCB1; SNF5) activity. Next steps could include developing SchLAP1 inhibitors. SciBX 6(42); doi:10.1038/scibx.2013.1187 Published online Oct. 31, 2013	Patent application filed; WaferGen Biosystems Inc. has a nonexclusive license to create commercial research assays for the detection of lncRNAs, including SchLAP1; GenomeDx Biosciences Inc. has licensed lncRNAs, including SchLAP1, for the molecular analysis of clinical prostate cancer samples	Prensner, J.R. <i>et al. Nat. Genet.</i> ; published online Sept. 29, 2013; doi:10.1038/ng.2771 Contact: Arul M. Chinnaiyan, University of Michigan, Ann Arbor, Mich. e-mail: arul@med.umich.edu
Small cell lung cancer (SCLC)	Unknown	Computational, cell culture and mouse studies identified marketed drugs that could be repurposed to treat SCLC. A bioinformatics approach was used to analyze a library of FDA-approved drugs screened against multiple cell types. In mouse and human lung cancer cell lines, five of the six top-scoring candidates from the screen inhibited viability of multiple SCLC lines but not non-SCLC (NSCLC) lines. In mouse allograft and xenograft models of SCLC, the three most potent compounds—imipramine, promethazine and bepridil—each inhibited tumor growth. Next steps include studies to understand why the targets of imipramine and related molecules induce cell death in neuroendocrine tumor cells. Imipramine is a generic tricyclic antidepressant. Promethazine is a generic antipsychotic. Bepridil is a generic calcium channel blocker. SciBX 6(42); doi:10.1038/scibx.2013.1188 Published online Oct. 31, 2013	Findings unpatented; bioinformatics approach licensed to NuMedii Inc.	Jahchan, N.S. <i>et al. Cancer Discov.</i> ; published online Sept. 26, 2013; doi:10.1158/2159-8290.CD-13-0183 Contact: Julien Sage, Stanford University, Stanford, Calif. e-mail: julsage@stanford.edu Contact: Atul J. Butte, same affiliation as above e-mail: abutte@stanford.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cardiovascular disease				
Coronary artery disease (CAD); myocardial infarction (MI)	Apolipoprotein D (APOD)	Mouse studies suggest APOD could help treat CAD and MI. In a mouse model of CAD caused by loss of <i>scavenger receptor class B member 1 (Scarb1)</i> and <i>apolipoprotein E (ApoE)</i> function, Apod expression in the heart was greater than that in CAD-free hearts. In a mouse model of MI, <i>Apod</i> knockout resulted in larger infarct areas than no knockout. In this model, hepatic overexpression of Apod decreased infarct size compared with no overexpression. Next steps could include testing APOD in additional animal models of CAD.	Patent and licensing status unavailable	Tsukamoto, K. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Sept. 30, 2013; doi:10.1073/pnas.1315986110 Contact: Monty Krieger, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: krieger@mit.edu
SciBX 6(42); doi:10.1038/scibx.2013.1189 Published online Oct. 31, 2013				
Dermatology				
Wounds	Myocardin-related transcription factor A (MKL1; MAL; MRTF-A)	Mouse studies suggest MRTF-A activation could help treat dermal wounds. In mice with dermal wounds, local application of an MRTF-A-stimulating isoxazole ring-containing small molecule decreased wound size compared with vehicle and accelerated wound closure. Next steps could include testing the compound in additional animal models of wound repair and investigating its mechanism of action.	Patent and licensing status unavailable	Velasquez, L.S. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Sept. 30, 2013; doi:10.1073/pnas.1316764110 Contact: Eric M. Small, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. e-mail: eric_small@urmc.rochester.edu Contact: Eric N. Olson, The University of Texas Southwestern Medical Center, Dallas, Texas e-mail: eric.olson@utsouthwestern.edu
SciBX 6(42); doi:10.1038/scibx.2013.1190 Published online Oct. 31, 2013				
Infectious disease				
HIV/AIDS	CD4	<i>In vitro</i> studies suggest addition of an <i>N</i> -linked glycan to the anti-CD4 mAb ibalizumab could help improve its ability to neutralize HIV-1 strains that are resistant to the antibody. Ibalizumab variants engineered with <i>N</i> -linked glycans on their light chains were synthesized. In a panel of 118 HIV-1 strains, 1 of the engineered variants neutralized all strains, including those resistant to unmodified ibalizumab. Next steps could include IND-enabling studies for the <i>N</i> -linked glycan-modified ibalizumab. In 2007, TaiMed Biologics Inc. licensed ibalizumab from Roche's Genentech Inc. unit. The compound has completed Phase II testing to treat HIV infection, and the company has a second-generation ibalizumab variant developed in collaboration with The Rockefeller University in preclinical development.	Patent application filed; licensed to TaiMed Biologics	Song, R. <i>et al. Nat. Biotechnol.</i> ; published online Oct. 6, 2013; doi:10.1038/nbt.2677 Contact: David D. Ho, The Rockefeller University, New York, N.Y. e-mail: dho@adarc.org
SciBX 6(42); doi:10.1038/scibx.2013.1191 Published online Oct. 31, 2013				
Malaria	<i>Plasmodium falciparum</i> dihydroorotate dehydrogenase (PfDHODH)	Mouse and <i>in vitro</i> studies identified dihydrothiophenone-derived inhibitors of PfDHODH that could be useful for treating malaria. In <i>in vitro</i> assays, the lead compound inhibited PfDHODH with an IC ₅₀ of 6 nM and had more than 14,000-fold selectivity for the parasitic enzyme over the human homolog. In two <i>P. falciparum</i> strains, the lead compound inhibited parasite growth with IC ₅₀ values of 15 or 18 nM. In mice, the lead compound had 40% oral bioavailability. Next steps include additional preclinical testing of the lead inhibitors as antimalarial agents.	Patent application filed; available for licensing	Xu, M. <i>et al. J. Med. Chem.</i> ; published online Sept. 27, 2013; doi:10.1021/jm400938g Contact: Honglin Li, East China University of Science and Technology, Shanghai, China e-mail: hlli@ecust.edu.cn Contact: Yufang Xu, same affiliation as above e-mail: yfxu@ecust.edu.cn Contact: Lili Zhu, same affiliation as above e-mail: zhulfl@ecust.edu.cn
SciBX 6(42); doi:10.1038/scibx.2013.1192 Published online Oct. 31, 2013				

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Viral infection	Calcium channel voltage-dependent $\alpha 2/\delta$ subunit 2 (CACNA2D2)	<i>In vitro</i> and mouse studies suggest voltage-gated calcium channel inhibitors could help treat or prevent New World hemorrhagic fever triggered by arenaviruses. In human osteosarcoma cells, a small interfering RNA screen identified CACNA2D2 and other calcium channel subunits as host factors that contribute to infection by the Junin arenavirus. In mouse macrophages, knockout of <i>Cacna2d2</i> or inhibition of voltage-gated calcium channels prevented viral entry and infection. In mice, a generic CACNA2D2 inhibitor prevented systemic or intracranial infection and decreased viral titers compared with vehicle. Next steps include testing calcium channel inhibitors in additional animal models of arenavirus infection. SciBX 6(42); doi:10.1038/scibx.2013.1193 Published online Oct. 31, 2013	Findings currently unpatented; available for licensing	Lavanya, M. <i>et al. Sci. Transl. Med.</i> ; published online Sept. 25, 2013; doi:10.1126/scitranslmed.3006827 Contact: Susan R. Ross, University of Pennsylvania, Philadelphia, Pa. e-mail: rosss@mail.med.upenn.edu
Inflammation				
Inflammation	Not applicable	<i>In vitro</i> and rat studies identified nitroxyl (HNO)-donating aspirin derivatives that could help treat inflammation. In rats, a diazeniumdiolate-based, HNO-releasing aspirin derivative had anti-inflammatory activity comparable to that for aspirin but led to decreased stomach ulceration. Next steps include synthesizing additional compounds and further characterizing the compounds' mechanism of action. SciBX 6(42); doi:10.1038/scibx.2013.1194 Published online Oct. 31, 2013	Patent application filed; available for licensing	Basudhar, D. <i>et al. J. Med. Chem.</i> ; published online Sept. 17, 2013; doi:10.1021/jm400196q Contact: Katrina M. Miranda, The University of Arizona, Tucson, Arizona e-mail: kmiranda@email.arizona.edu
Musculoskeletal disease				
Musculoskeletal disease	Adenylate cyclase-stimulating G α protein (GNAS); glioma-associated oncogene homolog 1 zinc finger protein (GLI1)	<i>In vitro</i> and mouse studies suggest hedgehog pathway inhibitors could help treat heterotopic ossification caused by loss-of-function GNAS mutations. In mice, knockout of <i>Gnas</i> in mesenchymal progenitor cells upregulated hedgehog signaling compared with wild-type <i>Gnas</i> expression and induced heterotopic ossification. In the mouse model, knockout or pharmacological inhibition of <i>Gli1</i> , a hedgehog signaling effector, decreased heterotopic ossification compared with no <i>Gli1</i> knockout or inhibition. Next steps include testing hedgehog pathway antagonists in nongenetic preclinical models of heterotopic ossification and assessing their safety. SciBX 6(42); doi:10.1038/scibx.2013.1195 Published online Oct. 31, 2013	Patent application filed; available for licensing	Regard, J.B. <i>et al. Nat. Med.</i> ; published online Sept. 29, 2013; doi:10.1038/nm.3314 Contact: Yingzi Yang, National Institutes of Health, Bethesda, Md. e-mail: yingzi@mail.nih.gov
Neurology				
Alzheimer's disease (AD)	Microtubule-associated protein- τ (MAPT; TAU; FTDP-17)	Cell culture and mouse studies identified anti-TAU mAbs that could be useful for treating AD. TAU aggregation is thought to contribute to late-stage AD. In cell culture, anti-TAU mAbs prevented the spreading of TAU aggregation. In a mouse model of AD driven by Tau overexpression, intracerebroventricular administration of anti-Tau mAbs decreased Tau aggregation and increased cognitive function compared with no antibody. Next steps could include testing anti-TAU mAbs in other models of AD that feature TAU pathology. TauRx Pharmaceuticals Ltd. has LMTX, a small molecule inhibitor of TAU aggregation, in Phase III testing for AD. At least four other companies have TAU-targeted therapeutics in preclinical or Phase I development to treat AD. SciBX 6(42); doi:10.1038/scibx.2013.1196 Published online Oct. 31, 2013	Patent and licensing status undisclosed	Yanamandra, K. <i>et al. Neuron</i> ; published online Sept. 26, 2013; doi:10.1016/j.neuron.2013.07.046 Contact: David M. Holtzman, Washington University in St. Louis School of Medicine, St. Louis, Mo. e-mail: holtzman@neuro.wustl.edu Contact: Marc I. Diamond, same affiliation as above e-mail: diamondm@neuro.wustl.edu

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Parkinson's disease (PD)	Endonuclease G (ENDOG)	<i>In vitro</i> and <i>in vivo</i> studies suggest inhibiting ENDOG could help treat PD. In human neuroblastoma cells overexpressing α -synuclein (SNCA), ENDOG translocated from the mitochondria to the nucleus and caused DNA degradation and cell death, which could be blocked by small hairpin RNA knockdown of ENDOG. In nematodes, endog knockout decreased SNCA-induced dopaminergic neuron cell death compared with no knockout. In fruit flies, RNAi targeting EndoG decreased SNCA-induced mortality and motor function impairments compared with RNAi controls. Next steps include identifying a specific ENDOG inhibitor. SciBX 6(42); doi:10.1038/scibx.2013.1197 Published online Oct. 31, 2013	Patent application filed; available for licensing	Büttner, S. <i>et al.</i> <i>EMBO J</i> ; published online Oct. 15, 2013; doi:10.1038/emboj.2013.228 Contact: Frank Madeo, University of Graz, Graz, Austria e-mail: frank.madeo@uni-graz.at Contact: Stephan J. Sigrist, Free University of Berlin, Berlin, Germany e-mail: stephan.sigrist@fu-berlin.de
Various				
Cardiovascular disease; sickle cell disease (SCD)	Hemopexin (HPX); toll-like receptor 4 (TLR4)	Mouse studies suggest TLR4 blockade or recombinant HPX could help prevent SCD-associated acute chest syndrome. In a transgenic mouse model of SCD, infusion of hemin, a breakdown product of hemoglobin, amplified endogenous hemin and caused acute lung injury, respiratory failure and death. In the mouse model, a TLR4 antagonist or the recombinant hemin scavenger HPX prevented hemin-induced respiratory failure and death, whereas vehicle did not. Next steps could include testing TLR4 antagonists or recombinant HPX in additional animal models of SCD-associated acute chest syndrome. Eisai Co. Ltd.'s TLR4 inhibitor, E5564, is in Phase III testing to treat sepsis. At least three other companies have TLR4 inhibitors in Phase II testing or earlier to treat sepsis, autoimmune disorders or inflammatory diseases. SciBX 6(42); doi:10.1038/scibx.2013.1198 Published online Oct. 31, 2013	Patent and licensing status unavailable	Ghosh, S. <i>et al.</i> <i>J. Clin. Invest.</i> ; published online Oct. 1, 2013; doi:10.1172/JCI64578 Contact: Solomon Fiifi Ofori-Acquah, Emory University School of Medicine, Atlanta, Ga. e-mail: soforia@emory.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
Disease models			
<i>Additional sex combs like 1 (Asxl1)</i> -mutant mouse model of myelodysplastic syndrome (MDS)	Mice transplanted with bone marrow expressing a mutant form of <i>Asxl1</i> could model MDS and be used to identify and evaluate new treatments for the condition. Mutations in <i>ASXL1</i> are found in patients who have MDS, myeloproliferative neoplasms and various types of leukemia. In the model, mice received a bone marrow transplant of cells obtained from mice treated with 5-fluorouracil and transduced with a vector expressing a C-terminal-truncated mutant form of <i>Asxl1</i> . The transplanted mice recapitulated the hallmarks of MDS seen in patients, including multilineage myelodysplasia, pancytopenia and occasional progression to leukemia. Next steps include using the model to evaluate therapies to treat <i>ASXL1</i> -mutant MDS.	Unpatented; model available for licensing	Inoue, D. <i>et al. J. Clin. Invest.</i> ; published online Oct. 8, 2013; doi:10.1172/JCI70739 Contact: Toshio Kitamura, Institute of Medical Science, The University of Tokyo, Tokyo, Japan e-mail: kitamura@ims.u-tokyo.ac.jp
	SciBX 6(42); doi:10.1038/scibx.2013.1199 Published online Oct. 31, 2013		
<i>Methyl CpG binding protein 2 (MECP2; RTT)</i> -deficient neurons derived from human embryonic stem cells (hESCs)	<i>MECP2</i> -deficient neurons derived from hESCs could be useful for studying Rett syndrome and screening for therapeutics to treat the disease. Transcription activator-like effector nuclease (TALEN)-based genome editing was used to generate <i>MECP2</i> -deficient hESCs, which were then differentiated into neurons. <i>MECP2</i> -deficient neurons showed impairments in transcriptional activity, protein synthesis, neurite complexity and protein kinase B (PKB; PKBA; AKT; AKT1) and mammalian target of rapamycin (mTOR; FRAP; RAFT1) pathway signaling, whereas nondeficient isogenic neurons did not. In the <i>MECP2</i> -deficient neurons, activation of AKT and mTOR signaling rescued impairments in transcriptional activity, protein synthesis and neuronal complexity. Next steps could include using the neurons to evaluate therapeutic candidates to treat Rett syndrome.	Patent and licensing status unavailable	Li, Y. <i>et al. Cell Stem Cell</i> ; published online Oct. 3, 2013; doi:10.1016/j.stem.2013.09.001 Contact: Rudolf Jaenisch, Whitehead Institute for Biomedical Research, Cambridge, Mass. e-mail: jaenisch@wi.mit.edu
	SciBX 6(42); doi:10.1038/scibx.2013.1200 Published online Oct. 31, 2013		
Transgenic mouse model of early α -synuclein (SNCA) overexpression in Parkinson's disease (PD)	An SNCA-overexpressing transgenic mouse PD model could help define early defects in dopaminergic neurons and help evaluate candidate therapeutic strategies. The mice show 1.9-fold increased expression of human <i>SNCA</i> over endogenous levels at developmental stages and more closely mimic PD-relevant pathology compared with existing models. In these mice, high levels of <i>SNCA</i> resulted in a persistent decrease of dopamine release in the brain that occurred prior to the loss of dopamine neurons or onset of motor deficits. Next steps could include using the model to evaluate early intervention strategies for PD.	Patent and licensing status unavailable	Janezic, S. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Sept. 30, 2013; doi:10.1073/pnas.1309143110 Contact: Richard Wade-Martins, University of Oxford, Oxford, U.K. e-mail: richard.wade-martins@dpag.ox.ac.uk
	SciBX 6(42); doi:10.1038/scibx.2013.1201 Published online Oct. 31, 2013		

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Drug delivery			
DNA nanostructures for targeted drug delivery	<p>Nanostructures generated via unconventional assembly of DNA could enable targeted drug delivery or be used as imaging agents. Rolling circle replication and liquid crystallization of long DNA building blocks created stable structures called nanoflowers that did not rely on Watson-Crick base pairing. These structures resisted denaturation, dissociation at low concentrations and degradation by DNase 1 in human serum. In flow cytometry studies, aptamer-loaded complexes showed greater colocalization with cancer cells than with control cells. In cancer cell lines, doxorubicin-containing aptamer nanoflowers showed increased cytotoxicity compared with free doxorubicin. Next steps include process optimization and preclinical pharmacokinetic testing of the nanoflowers.</p> <p>SciBX 6(42); doi:10.1038/scibx.2013.1202 Published online Oct. 31, 2013</p>	Patent filed by the University of Florida; available for licensing	<p>Zhu, G. <i>et al. J. Am. Chem. Soc.</i>; published online Sept. 18, 2013; doi:10.1021/ja406115e Contact: Weihong Tan, University of Florida, Gainesville, Fla. e-mail: tan@chem.ufl.edu</p>
Drug platforms			
Functionalizing mAbs with a noncovalent peptide-binding site	<p>A peptide-binding site found on Erbitux cetuximab could be used to functionalize mAbs without disrupting their native antigen-binding properties. Crystal structure, biophysical and sequencing studies identified a unique site in the Fab framework of Erbitux that can noncovalently bind to an engineered peptide called a meditope. The binding site was grafted onto Herceptin trastuzumab. In HER2 (EGFR2; ErbB2; neu)⁺ human breast cancer cell lines, the modified trastuzumab bound with affinity comparable to that of unmodified trastuzumab and was also shown to bind with a meditope-Fc construct. Next steps include trying to improve the affinity of the meditope-mAb interaction and evaluating the use of meditope-enabled mAbs for research and therapeutic applications. Meditope Biosciences Inc. is developing meditope-enabled mAbs and corresponding meditope. Roche's Genentech Inc. unit markets Herceptin, a humanized mAb against HER2, to treat breast and gastric cancers. Eli Lilly and Co., Bristol-Myers Squibb Co. and Merck KGaA market Erbitux, a chimeric IgG1 mAb targeting epidermal growth factor receptor (EGFR), to treat colorectal cancer and head and neck cancer (<i>see Building meditope-enabled mAbs, page 1</i>).</p> <p>SciBX 6(42); doi:10.1038/scibx.2013.1203 Published online Oct. 31, 2013</p>	Patent applications filed by City of Hope; exclusively licensed to Meditope; available for partnering through Meditope	<p>Donaldson, J.M. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Oct. 7, 2013; doi:10.1073/pnas.1307309110 Contact: John C. Williams, Beckman Research Institute at City of Hope, Duarte, Calif. e-mail: jwilliams@coh.org</p>
Image-based high-content screening platform to survey large compound libraries	<p>A platform combining chemical screening with automated image acquisition and analysis could enable the identification of compounds that inhibit disease-associated signal transduction pathways. To demonstrate proof of concept for the approach, about 17,000 compounds were screened for their potential to interfere with the subcellular localization of a fluorescent Notch 1 (NOTCH1) reporter. The screen led to the isolation of two new γ-secretase inhibitors and FLI-06, a dihydropyridine that decreased protein trafficking. Next steps include applying the platform to other signaling pathways and further developing FLI-06.</p> <p>SciBX 6(42); doi:10.1038/scibx.2013.1204 Published online Oct. 31, 2013</p>	Patent filed covering use of FLI-06 and derivatives; not yet available for licensing	<p>Krämer, A. <i>et al. Nat. Chem. Biol.</i>; published online Sept. 29, 2013; doi:10.1038/nchembio.1356 Contact: Christoph Kaether, Fritz Lipmann Institute, Jena, Germany e-mail: ckaether@fli-leibniz.de</p>

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Intramyocardial injection of synthetic modified RNA (modRNA) encoding VEGF-A to expand and direct differentiation of cardiac progenitor cells	<p>Mouse studies suggest pulse-like expression of VEGF-A from modRNA could help regenerate cardiac tissue. In a mouse model of myocardial infarction, modRNA- and DNA-mediated expression of VEGF-A increased capillary density and decreased apoptosis and infarct size compared with vehicle. In the model, modRNA-mediated expression of VEGF-A increased long-term survival compared with that seen using vehicle. In contrast, DNA-mediated expression of VEGF-A decreased short-term survival. Next steps include testing this approach in larger animal models and humans.</p> <p>SciBX 6(42); doi:10.1038/scibx.2013.1205 Published online Oct. 31, 2013</p>	Patented; available for licensing	<p>Zangi, L. <i>et al. Nat. Biotechnol.</i>; published online Sept. 8, 2013; doi:10.1038/nbt.2682 Contact: Kenneth R. Chien, Karolinska Institute, Stockholm, Sweden e-mail: kenneth.chien@ki.se</p>
Reprogramming <i>in vivo</i> to generate totipotent induced pluripotent stem (iPS) cells	<p><i>In vivo</i> reprogramming in mice could be used to generate highly plastic iPS cells that can efficiently contribute to extraembryonic tissue such as placenta. Mice were engineered to express four doxycycline-inducible reprogramming factors: <i>Oct4</i>, <i>Sox2</i>, <i>Klf4</i> and <i>c-Myc</i> (<i>Myc</i>). In these mice, doxycycline led to the formation of iPS cells in multiple organs that originated from multiple cell types. The <i>in vivo</i>-generated iPS cells were more efficient at forming embryonic and extraembryonic tissues than embryonic stem cells or <i>in vitro</i>-generated iPS cells. Next steps include understanding the mechanism of <i>in vivo</i> reprogramming and why the resulting iPS cells show greater totipotency than other stem cell lines.</p> <p>SciBX 6(42); doi:10.1038/scibx.2013.1206 Published online Oct. 31, 2013</p>	<p>Patent application filed; available for licensing through the Botín Foundation Contact: Miriam Zeini, Botín Foundation, Madrid, Spain e-mail: mzeini@fundacionbotin.org</p>	<p>Abad, M. <i>et al. Nature</i>; published online Sept. 11, 2013; doi:10.1038/nature12586 Contact: Manuel Serrano, Spanish National Cancer Research Centre (CNIO), Madrid, Spain e-mail: mserrano@cnio.es</p>
Toll-like receptor 9 (TLR9) or TLR3 adjuvants to promote cancer vaccine-induced effector T cell expansion	<p>Mouse studies suggest TLR9 or TLR3 ligands can enhance cancer vaccine-induced effector T cell expansion. In mice, vaccines formulated with the TLR9 ligand CpG or the TLR3 ligand poly(I:C) expanded effector T cell populations more than vaccines formulated with other adjuvants or without adjuvant. In mouse xenograft models of melanoma, vaccination using TLR9 or TLR3 ligands as adjuvants prolonged survival and slowed tumor growth and increased effector T cell infiltration into tumors compared with vaccination using other adjuvants. Next steps include combining these adjuvants with other formulations to enhance the antitumor immune response and including an antigen delivery system to improve spatiotemporal control over immune system activation.</p> <p>SciBX 6(42); doi:10.1038/scibx.2013.1207 Published online Oct. 31, 2013</p>	Unpatented; licensing status not applicable	<p>Perret, R. <i>et al. Cancer Res.</i>; published online Sept. 18, 2013; doi:10.1158/0008-5472.CAN-13-0875 Contact: Rachel Perret, University of Lausanne, Epalinges, Switzerland e-mail: rachel.perret@unil.ch</p>
Imaging			
Copper complex-based imaging agents to diagnose Alzheimer's disease (AD)	<p>Studies in mice and human samples suggest copper complex-based imaging agents could help diagnose AD. In wild-type mice, microPET imaging showed that β-amyloid (Aβ) plaque-targeted thiosemicarbazone-pyridylhyseoxazine tetradentate ligands form a complex with a copper isotope and can cross the blood brain barrier and quickly dissipate when Aβ plaques are absent. In brain tissue samples from patients with AD, the ligands bound to antibody-labeled Aβ plaques, whereas they did not bind to tissue from healthy controls. Next steps could include testing the imaging agents in mouse models of AD.</p> <p>SciBX 6(42); doi:10.1038/scibx.2013.1208 Published online Oct. 31, 2013</p>	Patent and licensing status unavailable	<p>Hickey, J.L. <i>et al. J. Am. Chem. Soc.</i>; published online Sept. 26, 2013; doi:10.1021/ja4057807 Contact: Paul S. Donnelly, The University of Melbourne, Melbourne, Victoria, Australia e-mail: pauld@unimelb.edu.au</p>

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Markers			
Gene expression signature to predict outcome in patients with idiopathic pulmonary fibrosis (IPF)	<p>Studies in patient samples identified a gene expression signature that could help predict outcomes in patients with IPF. In peripheral blood mononuclear cells (PBMCs) from 45 patients with IPF, microarray analysis identified 7 genes whose high expression was associated with shorter transplant-free survival and 45 genes whose low expression was associated with shorter transplant-free survival. In a replication cohort of 75 patients, cluster analysis based on the 52-gene signature showed that a high-risk group had median transplant-free survival of 1.62 years, whereas a low-risk group had a median of 3.44 years. In the patient cohorts, quantitative RT-PCR analysis of 4 genes from the 52-gene panel was sufficient to predict poor outcomes as measured by decreased transplant-free survival. Next steps include validating the gene signature in a larger patient cohort.</p> <p>SciBX 6(42); doi:10.1038/scibx.2013.1209 Published online Oct. 31, 2013</p>	Patent application filed; licensing details available from The University of Chicago and the University of Pittsburgh	<p>Herazo-Maya, J.D. <i>et al. Sci. Transl. Med.</i>; published online Oct. 2, 2013; doi:10.1126/scitranslmed.3005964</p> <p>Contact: Naftali Kaminski, Yale School of Medicine, New Haven, Conn. e-mail: naftali.kaminski@yale.edu</p>

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