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## VEGF reflects on itself

By **Tim Fulmer**, Senior Writer

North American researchers have chemically synthesized a D-protein ligand that blocked the binding of VEGF to its receptor *in vitro*.<sup>1</sup> **Reflexion Pharmaceuticals Inc.** has licensed the compound and thinks it could have better stability and bioavailability than anti-VEGF antibodies that are L-isomer proteins. The company is optimizing its molecule before doing head-to-head comparisons with Lucentis and Avastin in animal models of age-related macular degeneration.

Protein and peptide molecules expressed in all organisms, including recombinant antibodies produced in bacteria, occur only as the left-handed L-isomer and never as the mirror-image, right-handed D-isomer.

Although biologics developers generally have not been concerned with that distinction, protein therapeutics based on the D-isomer should have greater stability and serum half-life than the L-isomer because the D-isomer is not recognized by the body's proteases. The ability to resist proteolytic degradation also raises the possibility of delivering D-isomers orally.

The open question is whether a D-protein therapeutic can be synthesized and optimized to hit its target. To date, researchers have studied only small D-peptides<sup>2</sup> and not D-proteins that potentially consist of hundreds of amino acids and could be quite challenging to synthesize and optimize.

In 2009, Stephen Kent, Sachdev Sidhu and Dana Ault-Riché founded Reflexion with the goal of developing D-protein-based therapeutics. As initial proof of principle, they decided to design a D-protein that bound VEGF and prevented it from binding the VEGF receptor (VEGFR).

Kent is a professor of chemistry at **The University of Chicago**. Sidhu is co-investigator at the **Ontario Institute for Cancer Research** and associate professor at the **University of Toronto**. Ault-Riché is CEO of Reflexion.

They chose VEGF because it is a validated therapeutic target and would allow for a direct comparison between a D-protein VEGF antagonist and an antibody-based VEGF antagonist such as Avastin

**"D-Protein inhibitors enjoy a huge advantage over D-peptide inhibitors when it comes to targeting extracellular proteins such as receptors, as it is generally more difficult to design high-affinity small peptide antagonists of receptors."**

—**Wuyuan Lu**,  
**University of Maryland**  
**School of Medicine**

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bevacizumab. Moreover, Kent had experience working with VEGF. In 2011, he published in *Angewandte Chemie International Edition* the total chemical synthesis of the L-isomer of the 204-amino-acid growth factor.<sup>3</sup>

Avastin is marketed by **Roche**, its **Genentech Inc.** unit and **Chugai Pharmaceutical Co. Ltd.** to treat multiple solid cancers.

For their D-protein antagonist screening assay, Reflexion licensed technology from the **Massachusetts Institute of Technology's Whitehead Institute for Biomedical Research** that allowed them to screen ligand and protein libraries against the D-isomer of the target protein. The method, dubbed mirror image phage display, originally was developed by Peter Kim in the mid-1990s when he was a researcher at Whitehead.<sup>4,5</sup> Kim now is EVP and president of **Merck & Co. Inc.**'s Merck Research Laboratories.

Mirror image phage display uses the D-isomer of the target protein to screen a library of L-isomer ligands. The top hits are then chemically converted to D-isomers.

The final D-isomer ligand binds the original protein with high affinity and specificity.

Reflexion synthesized D-VEGF using the chemical synthesis method described in the 2011 paper. Next, the researchers used D-VEGF to screen a large library of L-protein ligands. The scaffold for the library was derived from the B1 domain of streptococcal protein G (GB1), a 56-amino-acid protein that is known to interact strongly with VEGF and is sufficiently stable to be used in a phage display assay.

Following multiple screening rounds, the L-protein ligand that bound D-VEGF with the highest affinity was chemically converted to the mirror image D-protein ligand, which was predicted to bind VEGF with high affinity.

Indeed, the D-protein blocked the binding of VEGF to VEGFR *in vitro*. Moreover, protein X-ray crystallography studies showed that

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the D-protein bound the region of VEGF that interacts with VEGFR, confirming the D-protein was an antagonist of the VEGF-VEGFR interaction.

The findings were published in the *Proceedings of the National Academy of Sciences*.

The key difference between the *PNAS* findings and prior work in the field “is that the authors constructed a protein library for their screen, as opposed to a peptide library, thus obtaining a D-protein inhibitor,” Wuyuan Lu told *SciBX*. He is professor of biochemistry and molecular biology at the **University of Maryland School of Medicine**.

“D-Protein inhibitors enjoy a huge advantage over D-peptide inhibitors when it comes to targeting extracellular proteins such as receptors, as it is generally more difficult to design high-affinity small peptide antagonists of receptors,” said Lu. The reason, he said, is that thermodynamic factors generally cause small peptides to interact weakly with receptors.

Earlier this year, Lu and colleagues published in the *Journal of Medicinal Chemistry* that mirror image phage display identified a high affinity D-peptide antagonist of the cancer target mdm2 p53 binding protein homolog (MDM2; HDM2).<sup>6</sup>

D-Protein inhibitors also have a potential cost advantage over antibodies because they are produced as a totally synthetic product, unlike biologics that require cell culture, said Michael Kay, assistant professor of biochemistry at **The University of Utah School of Medicine**.

Kay and colleagues have developed D-peptide HIV entry inhibitors that are exclusively licensed to **Navigen Pharmaceuticals Inc.**<sup>7,8</sup> The company’s lead D-peptide inhibitor, PIE12-trimer, is in preclinical development to prevent and treat HIV.

Because the *PNAS* paper has no *in vivo* data, “obviously a lot more needs to be done to study the PK, PD and toxicity profiles” of the D-protein antagonist, said Lu.

Also important will be “studies in suitable transgenic animal models,” added Dieter Willbold, professor of physical biology at the **Heinrich Heine University of Duesseldorf**. Willbold and colleagues have shown that an orally available D-peptide ligand of  $\beta$ -amyloid (A $\beta$ ) reduced pathology and improved behavior in transgenic mouse models of Alzheimer’s disease (AD).<sup>9</sup>

### The right-hand path

Reflexion “has already produced affinity-matured, next-generation molecules based on the initial lead in the *PNAS* article,” Sidhu told *SciBX*. He added that the best antagonists “will be validated in cell-based assays to assess inhibition of VEGF activity in physiologically relevant systems and also tested in animal models of age-related macular degeneration and cancer, in comparison with Lucentis and Avastin.”

The anti-VEGF mAb Lucentis ranibizumab is marketed by Roche, Genentech and **Novartis AG** to treat age-related macular degeneration (AMD) and diabetic macular edema (DME).

Reflexion will also test its D-protein antagonists for immunogenicity. “We believe the D-amino-acid nature of our molecules should significantly reduce immunogenicity, since our molecules are resistant

to proteolysis and cannot be recognized by MHC,” said Sidhu. The major histocompatibility complex (MHC) plays a central role in triggering the host immune response.

To help carry out the animal testing, Reflexion is collaborating with Calvin Kuo, associate professor of medicine at the **Stanford University School of Medicine**. Kuo has expertise in animal models of angiogenesis, said Ault-Riché.

Sidhu said he is assembling a large panel of diverse scaffolds that will expand the power of the mirror image phage display technology.

Reflexion also is developing a D-protein compound to treat the orphan disease lymphangioliomyomatosis (LAM), which causes the lungs of women in their thirties and forties to develop cysts that destroy lung function.

“Studies suggest that LAM patients could benefit from a compound that antagonizes the VEGF-D homolog,” said Ault-Riché. “We should be able to do mirror image phage display much like what we did in the *PNAS* paper to identify D-protein antagonists of VEGF-D,” he said.

The compounds in the *PNAS* paper are covered by patents and are available for licensing from Reflexion.

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### COMPANIES AND INSTITUTIONS MENTIONED

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**Genentech Inc.**, South San Francisco, Calif.  
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**Massachusetts Institute of Technology**, Cambridge, Mass.  
**Merck & Co. Inc.** (NYSE:MRK), Whitehouse Station, N.J.  
**Navigen Pharmaceuticals Inc.**, Salt Lake City, Utah  
**Novartis AG** (NYSE:NVS; SIX:NOVN), Basel, Switzerland  
**Ontario Institute for Cancer Research**, Toronto, Ontario, Canada  
**Reflexion Pharmaceuticals Inc.**, San Francisco, Calif.  
**Roche** (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland  
**Stanford University School of Medicine**, Palo Alto, Calif.  
**The University of Chicago**, Chicago, Ill.  
**University of Maryland School of Medicine**, Baltimore, Md.  
**University of Toronto**, Toronto, Ontario, Canada  
**The University of Utah School of Medicine**, Salt Lake City, Utah  
**Whitehead Institute for Biomedical Research**, Cambridge, Mass.

# This is (a diagnostic) spinal tap

By Michael J. Haas, Senior Writer

A team led by **KineMed Inc.** researchers has shown that measuring the kinetics of CNS proteins in cerebrospinal fluid could help diagnose Parkinson's disease and amyotrophic lateral sclerosis.<sup>1</sup> The company is now exploring the utility of the approach to diagnose multiple neurodegenerative diseases and monitor treatment responses.

Neurons in the brain synthesize and secrete a range of proteins that move along the microtubule structures of axons to the nerve endings—a process known as axonal transport. There, the proteins enter extracellular fluids, including cerebrospinal fluid (CSF).

Multiple studies have suggested links between disruptions in axonal transport and PD, ALS, Alzheimer's disease (AD) and Huntington's disease (HD).<sup>2-8</sup> However, the molecular mechanisms underlying those disruptions are poorly understood, largely due to a lack of tools for studying axonal transport *in vivo*.

To build out the toolbox, the KineMed team turned to a method of deuterium (<sup>2</sup>H) labeling it had previously developed to investigate the effects of paclitaxel on microtubule structures in cancer cells.<sup>9</sup> In that study, the team administered heavy water (<sup>2</sup>H<sub>2</sub>O) to cells and animal models to measure the incorporation of deuterium into tubulin dimers and polymers and thus track changes in microtubule structure and function.

In its latest study, the team administered heavy water to disease models of PD and ALS and to patients with PD and collected CSF samples via lumbar puncture for analysis of selected deuterium-labeled proteins.

Mouse models received heavy water orally and/or by intraperitoneal injection and underwent lumbar punctures once daily for up to 10 days. Patients and healthy subjects received heavy water orally and lumbar punctures no more than 4 times over the course of 40 days.

The team analyzed the CSF samples with mass spectrometry to determine the concentrations of four deuterium-labeled cargo proteins known to rely on axonal transport: soluble amyloid precursor protein (APP), chromogranin B (CHGB), neuregulin 1 (NRG1) and  $\alpha$ -synuclein (SNCA). After plotting the concentration of each protein as a function of time, the team calculated the rates of appearance and disappearance for each protein in CSF.

Finally, for all proteins the researchers compared the protein appearance and disappearance rates in the disease model or patient with the rates in the corresponding control. Slower rates indicated a disease-related delay in the axonal transport of that protein.

For example, in healthy mice receiving intracerebroventricular infusions of nocodazole—a microtubule-destabilizing agent that disrupts axonal transport—the rates for Chgb and Nrg1 were lower than those in vehicle-treated controls, thus confirming that the method could detect changes in axonal transport.

The team also found that in PD mouse models and patients, the rates for three of the proteins—SNCA, CHGB and soluble APP—were lower than those in healthy controls. In mouse models of ALS, the rates for a different trio of proteins—Nrg1, Chgb and soluble App—were lower than those in healthy controls.

Additional experiments showed that the neuronal synthesis rates of the four proteins in the nocodazole-treated mice and the two disease models were similar to synthesis rates in the respective controls. This provided additional evidence that the method was measuring changes in axonal transport—not neuronal production—of the proteins, the team wrote in its report in *The Journal of Clinical Investigation*.<sup>1</sup>

The degree of deuterium enrichment of a protein also could reveal whether or not its axonal transport was disrupted, team coleader Marc Hellerstein told *SciBX*. By measuring the amount of deuterium incorporation in a protein that appeared in the CSF at a particular time point, the team could determine how long after heavy water administration the protein had been synthesized in neurons—and thus estimate how long it had taken the new protein to reach the CSF, he said.

“We knew the only factor that could explain why it took a week or so between synthesis in neurons and detection in CSF was axonal transport,” said Hellerstein.

Hellerstein is cofounder of KineMed and chairman of its scientific advisory board. He also is a professor at the **University of California, Berkeley** and professor of medicine at the **University of California, San Francisco's San Francisco General Hospital**.

Patrizia Fanara, team coleader and KineMed's VP of neuroscience, added that deuterium labeling is good for measuring slow processes like axonal transport because heavy water diffuses through living tissues over the course of many days. This means the heavy water remains in tissues and continues to label newly synthesized neuronal proteins with deuterium over the time period required to measure axonal transport rates.

She added, “It is also very easy to administer heavy water in outpatient clinical settings as we did in this study.”

The team included researchers from the **McLaughlin Research Institute for Biomedical Sciences** and the **University of Osnabrueck**.

“This looks exactly like the kind of good diagnostic tool we need in the difficult space of neurodegenerative disease,” said Rémy Luthringer, **Mind-NRG** board member and venture partner at **Index Ventures**.

The link between neurodegenerative disease and deficits in axonal transport of cargo proteins was already known, “but now we have a way to systematically assess axonal transport,” he said.

Luthringer wanted to know whether the axonal transport kinetics of cargo proteins change during the progression of a neurodegenerative disease. If so, the KineMed approach could enable early diagnosis—for example, before the onset of motor symptoms in PD—and thus guide treatment decisions, he said.

Barbara Tate, VP at **Satori Pharmaceuticals Inc.**, was less sanguine about the approach's utility as a diagnostic because the team tested it only in animals and patients with established disease. “I didn't see anything in the study to suggest the approach could be used to diagnose neurodegenerative disease before current clinical criteria are met” and significant brain damage already has occurred, she said.

Instead, she said, the approach was better suited for monitoring disease progression and treatment responses.

“Validation of this approach for monitoring treatment responses in patients would first require a therapeutic that modifies axonal transport, which in turn would require a valid marker for that process,” Tate said. “Thus, the approach is a long way from establishing clinical markers that regulatory agencies would accept for compound registration.”

Nevertheless, she said the approach could be useful for monitoring responses in preclinical studies.

Indeed, a problem with neurodegenerative disease models is that behavior is often used to measure cognition, said Jo Ann Dumin, principal research investigator at Satori. “This can result in a lot of false positives, and the measures don’t always translate to humans. So the possibility of having a biochemical marker in CSF as a surrogate for cognition in preclinical models is intriguing.”

She added, “Down the road, KineMed’s approach might have similar utility in patients because the tools for measuring cognition in humans are not always precise either.”

For instance, Dumin said, it would be interesting to see whether the approach could measure microtubule-associated protein- $\tau$  (MAPT; TAU; FTDP-17) and TAU-related proteins in CSF to follow progression and treatment response in patients with AD.

Tate agreed, noting that such biochemical markers—even those not accepted by regulatory agencies—could help determine whether a therapy was on the right track in the clinic. “If a company could use a biochemical marker to get an early read of therapeutic response in a small patient population, that could save a lot of time and money,” she said.

This year, Satori hopes to submit an IND for SPI-1865, a  $\gamma$ -secretase modulator, to treat AD.

### Heavy mettle

Another question is whether the technique can help identify subsets of neurons that are involved in neurodegenerative diseases.

Luthringer wanted to see KineMed’s approach combined with MRI or other readouts that could link altered axonal transport to anatomical or functional changes in specific areas of the brain. “That could help us better understand disease progression and assess response to specific therapies,” he said.

Fanara agreed and said the team’s preclinical findings suggest the axonal transport kinetics of a cargo protein may be specific to differing populations of neurons in each neurodegenerative disease. Thus, she said, it might eventually be possible to diagnose a disease by detecting altered axonal transport kinetics of cargo proteins in specific classes of neurons.

Additionally, “if we can link disruptions in axonal transport of a given target protein to a particular subset of neurons, we might gain insights into disease pathogenesis and progression,” Hellerstein said.

“It might be difficult to find patients willing to submit to a lumbar puncture” for diagnostic or monitoring purposes, cautioned Luthringer. “To get clinicians to recommend such an invasive procedure and to get patients to accept it, you would need to show it has some real benefit compared with noninvasive techniques.”

Dumin also said there might be diurnal variations in the levels of the proteins in CSF. “One might expect a marker of synaptic activity to show changes according to the time of day,” she said. “Also, the results could vary depending on where in the spine you take the sample.”

Nevertheless, Luthringer said the technique seemed equally applicable to preclinical and clinical studies, and “we are now thinking about using this method in our own studies at Mind-NRG.”

**“If a company could use a biochemical marker to get an early read of therapeutic response in a small patient population, that could save a lot of time and money.”**

—Barbara Tate,  
Satori Pharmaceuticals Inc.

Mind-NRG’s NRG-101, the extracellular fragment of the  $\beta$ 1 isoform of NRG1, is in preclinical development to treat PD and schizophrenia.

Fanara said KineMed is investigating the potential of the approach to diagnose and monitor treatment responses in PD, ALS, AD and HD.

The company has also begun a cross-sectional study in patients with PD—funded by **The Michael J. Fox Foundation for Parkinson’s Research** (MJFF)—to see “whether there is any

meaningful relationship between the degree of axonal transport deficit and severity of disease,” she said. The study will also follow the patients over time to look for associations between the degree of axonal transport deficit and progression of disease that could have prognostic value.

Additionally, KineMed is looking for other cargo proteins that rely on axonal transport as potential markers of neurodegenerative disease.

“We hope that tracking additional cargo proteins will reveal whether decreased axonal transport is specific for certain classes of neurons rather than universal” in a given disease, she said.

KineMed has exclusively licensed a portfolio of patents and patent applications covering the technology described in the *JCI* study from UC Berkeley.

The company is seeking corporate collaborators interested in applying the technology to the preclinical and clinical development of therapies to treat neurological disorders, Fanara said.

In 2008, KineMed received a \$700,000 grant from MJFF to fund the PD patient studies reported in the *JCI* paper. In June, MJFF awarded KineMed an additional \$1.2 million to continue developing the technology as a PD diagnostic.

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### COMPANIES AND INSTITUTIONS MENTIONED

**Index Ventures**, Geneva, Switzerland

**KineMed Inc.**, Emeryville, Calif.

**McLaughlin Research Institute for Biomedical Sciences**, Great Falls, Mont.

**The Michael J. Fox Foundation for Parkinson’s Research**, New York, N.Y.

**Mind-NRG**, Geneva, Switzerland

**San Francisco General Hospital**, San Francisco, Calif.

**Satori Pharmaceuticals Inc.**, Cambridge, Mass.

**University of California, Berkeley**, Calif.

**University of California, San Francisco**, Calif.

**University of Osnabrueck**, Osnabrueck, Germany

# Cracking ENCODE

By Lev Osherovich, Senior Writer

Results from the Encyclopedia of DNA Elements consortium have provided the first systematic and comprehensive look at how gene expression is regulated in humans. Although the data provide the most detailed picture yet of the human genome since its complete sequencing over a decade ago, the new information—much like the initial sequencing of the genome—has no immediate application to drug discovery. Rather, the data provide researchers with a more focused starting point for formulating new hypotheses about which targets to pursue.

A decade ago, the Encyclopedia of DNA Elements (ENCODE) consortium set out to catalog the function of the entire genome, not just the protein-coding portions previously thought to be the most important parts. The consortium used a variety of DNA- and RNA-profiling techniques on 147 human cell and tissue types to construct a database of complex genetic interactions that regulate the activity of genes.

The consortium's first-pass analysis of the genome, published last week in 32 papers across 6 journals, garnered headlines in the press for explaining the function of noncoding or 'junk' DNA in coordinating gene expression. Although regulatory functions of noncoding DNA have been observed previously, the extent to which these sequences

influence gene expression was a surprise.

However, gauging the true significance of noncoding DNA in disease will require years of old-fashioned experimental validation. In fact, the consortium uncovered considerably more information about genome interactions than just the junk DNA data that grabbed headlines.

A case in point is a study by **University of Washington** researchers on the regulatory functions of genomic regions

previously implicated in disease.<sup>1</sup> The study, arguably the most disease-relevant of the 32 papers published, showed that many noncoding DNA markers flagged in previous genomic studies of disease may control the expression of distant genes rather than of near neighbors, as is generally thought.

For academics, the entire set of findings provides a window into the complex structure of the genome. For industry, the findings are likely to spark a re-examination of which genes are truly regulated by noncoding regions identified in genomewide association (GWA) studies of common cardiovascular, metabolic, neurological and autoimmune diseases.

Collectively, the ENCODE studies paint a picture of how the genome's noncoding DNA—up to 80% of total DNA—coordinates the production of protein-coding mRNA.

The ENCODE data “rewrites the way we think about the genome,”

said Philip Gregory, CSO and VP of research at **Sangamo BioSciences Inc.** “Even the people who were into transcriptional regulation are surprised by the extent to which the genome controls its own gene expression profile.”

Companies that stand to gain the most from the discoveries are those with knockdown technologies that can rapidly screen for biological effects of modulating the genes that the ENCODE consortium identified as key disease players.

## Long-distance runaround

The University of Washington team, led by ENCODE consortium member John Stamatoyannopoulos, combined data from prior GWA studies and ENCODE's new information about the genome's physical interactions to predict which genes are central to disease.

The ENCODE study “gives us a framework for comprehensively analyzing the epigenetic basis of disease traits,” said Stamatoyannopoulos, associate professor of genome sciences and medicine.

His team used an *in vitro* chromosome-mapping technique to identify binding sites for transcriptional regulators throughout the genomes of 349 cell and tissue types from healthy individuals.

The group then compared the map of these regulatory sequences with 5,654 SNPs in noncoding regions drawn from 207 GWA studies. These SNPs were previously identified as hereditary factors in at least one disease.

Finally, the researchers used a physical mapping technique to identify gene promoters that were most likely to be activated by the proteins that bound to the SNP sites.

When these three data sets were superimposed, the resulting map pointed to the regions of the genome likely to be regulated by the disease-associated SNPs.

The surprise came from comparing the locations of disease-linked SNPs and their target genes. Previously, the assumption was that most regulatory DNA sequences affected the expression of nearby genes. Instead, the Stamatoyannopoulos team found that many disease-linked SNPs directly affected the expression of distant genes (*see Figure 1, “Rethinking disease markers”*).

Stamatoyannopoulos suspects this long-range regulation occurs because of physical interactions between the SNPs and their target genes across the complex 3D structure of chromatin.

“People had assumed that if a SNP is near a gene, maybe it's affecting a nearby gene,” said Stamatoyannopoulos. “But we found that regulatory DNA is controlling genes that are located 10–12 genes away.”

Results were published in *Science*. The raw data from the study are freely available, and Stamatoyannopoulos has filed patents on some of the analytical methods used in the study. The patents are available for licensing.

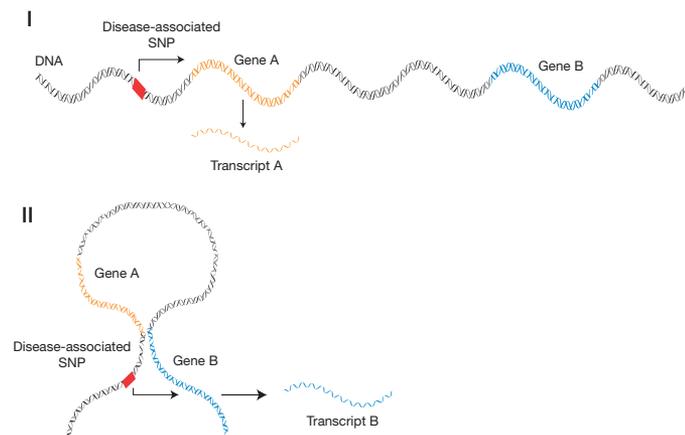
Other analyses by the ENCODE consortium were simultaneously published last week in 31 other papers in *Nature*, *Genome Research*, *Genome Biology*, *BMC Genetics*, *Cell* and *Science*.

## SNP off the old block

The findings suggest a slew of new potential players in many disease categories, but proving those proteins are bona fide targets will require

“As opposed to just relying on inferred causal connections from gene expression, these data help us to identify the specific proteins that are responsible for the biological effects that we observed.”

—Eric Schadt,  
Sage Bionetworks



**Figure 1. Rethinking disease markers.** Maurano *et al.* have uncovered evidence from genomewide association studies that the majority of noncoding DNA regions implicated in disease may exert their effects on distant genes, not adjacent ones as previously thought.

(I) SNPs in noncoding regions were thought to affect the expression of the nearest gene (Gene A), which was imputed to be involved in disease.

(II) Maurano *et al.* used data from the Encyclopedia of DNA Elements (ENCODE) consortium survey of genomic interactions to study the relationship between gene expression and disease-linked SNPs. The team concluded that noncoding SNPs typically affect the expression of distant genes (Gene B), not the nearest ones. The findings shift the focus of disease gene validation away from nearby genes toward distant genes.

independent experimental validation of the team's findings in cell culture and animal models.

Meanwhile, the results are forcing a rethink of previous GWA study findings.

For example, Stamatoyannopoulos' team compiled lists of genes that could be pivotal in various cancers and autoimmune, metabolic and neurodegenerative diseases. Many of these genes had been overlooked by previous GWA analyses because their immediate chromosomal environment did not have SNPs associated with disease.

Eric Schadt, professor and chair of genetics and genomic sciences and director of the Institute for Genomics and Multiscale Biology at **Mount Sinai School of Medicine**, said the findings provide a mechanism to explain prior observations about the complex regulation of gene expression.

Schadt is cofounder of **Sage Bionetworks**, a not-for-profit systems biology institute that spun out of **Merck & Co. Inc.**'s shuttered Rosetta Inpharmatics unit in 2009.

In 2008, Schadt's team reported results from Rosetta's comprehensive analysis of gene expression that pointed to central players in metabolic disease.<sup>2</sup> Schadt said Stamatoyannopoulos' findings identify the likely regulatory sites and the transcription factors that control expression of those genes.

"As opposed to just relying on inferred causal connections from gene expression, these data help us to identify the specific proteins that

are responsible for the biological effects that we observed," said Schadt. "These data will help us to identify causal variants and identify the proteins involved in the changes in gene expression that we identified as being important in disease."

The new findings also will be useful in helping to winnow the results of GWA studies down to the most critical players in disease. GWA studies are designed to find relatively common genetic variants that each contribute modestly to disease risk.

The goal of GWA studies is to gain insights into disease mechanisms, but this has proven difficult to do because so many GWA hits are in noncoding DNA with nonobvious biological effects.<sup>3</sup>

Stamatoyannopoulos said industry has largely steered clear of GWA studies because it has been hard to understand what disease-associated SNPs actually do.

"The traditional disease target approach is to see what's upregulated and downregulated and go after that," said Stamatoyannopoulos. "Now that there's a real way to connect these SNPs to targets in the genome," it should be possible to uncover the downstream genes that are the true drivers of disease.

### From hit to target

The challenge now is to show that hitting the regulatory elements and their target genes implicated by Stamatoyannopoulos' study can affect disease.

Gregory thinks Sangamo's zinc finger nuclease technology could be useful for studying the effect of tweaking the regulatory sites identified in Stamatoyannopoulos' study.

The company's technology is "capable of introducing mutations that change regulatory elements" identified by Stamatoyannopoulos' team, he said. "This would allow us to determine which of these are truly causative in disease."

Sangamo is collaborating with Stamatoyannopoulos to study how distant regulatory elements influence transcription of globin genes, which are misregulated in thalassemias, a common class of blood disorders.

Targeting the genes controlled by the disease-associated regulatory regions is a greater challenge because it is not immediately clear which genes to focus on and how to modulate their activity.

Moreover, many of the key genes identified by the Washington team are transcription factors, which are hard to drug.

Tim Harris, SVP of translational medicine and biochemistry at **Biogen Idec Inc.**, said companies will be poring over the data from the ENCODE consortium and from Stamatoyannopoulos' study to identify targetable candidate regions.

"The most immediate effect on drug discovery will be for companies that can look at these control regions to see how direct interference with them might affect disease," said Harris. He noted that companies using RNA interference technologies are well positioned to rapidly validate new targets suggested by the studies.

"Companies like **Alnylam Pharmaceuticals Inc.** and **Isis Pharmaceuticals Inc.**, which are interested in antisense approaches, will be able to make more of the data more immediately than most other people," said Harris.

Biogen Idec and Isis are developing preclinical antisense candidates for a range of neurological diseases.

(Continues on p. 8)

# Probing for a point-of-care TB test

By Kai-Jye Lou, Staff Writer

Existing tests to screen patients for tuberculosis are impractical in the point-of-care setting as they are either too slow, lack sensitivity or require specialized lab equipment.<sup>1,2</sup> Researchers at **Stanford University** and **Texas A&M University** have synthesized highly sensitive class A  $\beta$ -lactamase-specific fluorogenic probes that could potentially address all three shortcomings.<sup>3</sup> **Global BioDiagnostics Corp.** has in-licensed the technology.

Currently, there is no rapid and accurate point-of-care diagnostic for TB infection. Sputum smear microscopy is one of the quickest and simplest methods to screen patients but has low sensitivity, which leads to a high false-negative rate. Culture-based techniques are the gold standard but require several weeks before results are available due to the slow growth rate of *Mycobacterium tuberculosis*.

Culture methods also require lab equipment and facilities to grow the bacteria.

Newer entrants into the TB testing market include nucleic acid-based strategies that are more sensitive than smear microscopy and can provide results within two hours. However, such tests require expensive, specialized lab equipment and thus are hard to deploy in resource-limited settings. Moreover, these tests do not assay bacterial viability, which would be important for assessing treatment response.

A joint research group co-led by Jianghong Rao and Jeffrey Cirillo has been working on fluorogenic probes that could enable rapid and accurate detection of live *M. tuberculosis* in clinical samples without the need for specialized lab equipment or facilities.

Rao is an associate professor of radiology and chemistry at Stanford, and Cirillo is a professor in the Department of Microbial and Molecular Pathogenesis at the **Texas A&M Health Science Center**.

*M. tuberculosis* expresses class A  $\beta$ -lactamase (blaC), an enzyme whose hydrolyzing activity the researchers sought to exploit as the mode of activation for their fluorogenic probes.

$\beta$ -Lactamases (LACTBs) hydrolyze the  $\beta$ -lactam ring found in some classes of antibiotics, including penicillins and cephalosporins. Bacterial

strains that produce the enzyme resist  $\beta$ -lactam antibiotics.

In 2010, the research group reported a series of cephalosporin-based probes that fluoresce when hydrolyzed by  $\beta$ -lactamase.<sup>4</sup> These probes enabled the detection of *M. tuberculosis* with high sensitivity but lacked specificity for blaC. As a result,  $\beta$ -lactamases from other bacterial strains also caused the probe to emit a fluorescent signal. Moreover, the probes were slowly hydrolyzed by  $\beta$ -lactamase and required a few hours before the fluorescent signal was readily detectable.

Now, the researchers have synthesized a new series of cephalosporin-based probes with high specificity for blaC and improved kinetics that generate a detectable fluorescent signal within minutes of exposure to the enzyme.

*In vitro*, probes exposed to blaC showed 100- to 200-fold greater fluorescence than in the absence of blaC. The lead probe showed more than 1,000-fold higher selectivity for blaC over a closely related class A  $\beta$ -lactamase that is produced in other strains of bacteria.

The lead probe detected *M. tuberculosis* in unprocessed sputum samples containing as few as 10 colony-forming units and reported a positive result within 10 minutes. Assessments were made using a free luminescence mapping software application for smartphones and photos taken with the smartphone camera.

Results were published in *Nature Chemistry*.

“Our current probes significantly cut the detection time and can be used with unprocessed clinical samples,” said corresponding author Rao. “Using blaC as a detection marker is novel since other methods use DNA or serological markers.”

“The chemical probes have low manufacturing cost, show high sensitivity and specificity for the TB enzyme and react with the enzyme in a very rapid manner,” added Michael Norman, CEO and cofounder of Global BioDiagnostics. “These properties make the probes attractive for the development of a rapid and cost-effective test that could be used in the point-of-care setting to allow doctors to make a diagnosis of TB when they are still in contact with the patient.”

“This study offers early proof of concept that an enzyme expressed by *Mtb* could be detected in sputum samples,” added Madhukar Pai, associate professor in the Department of Epidemiology, Biostatistics and Occupational Health at **McGill University**. “If a simple enzyme detection test can work, and can be done without major investments in equipment

(Continues on p. 9)

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(Continued from “Cracking ENCODE,” p. 7)

Biogen Idec and Alnylam have a collaborative research agreement to discover RNA interference-based therapeutics for progressive multifocal leukoencephalopathy (PML). Last month, Biogen Idec partnered with **Regulus Therapeutics Inc.** to identify microRNA biomarkers in blood from patients with multiple sclerosis (MS). Regulus is a joint venture between Alnylam and Isis.

Osherovich, L. *SciBX* 5(36); doi:10.1038/scibx.2012.945  
Published online Sept. 13, 2012

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**Contact:** John A. Stamatoyannopoulos, University of Washington, Seattle, Wash.  
e-mail: [jstam@uw.edu](mailto:jstam@uw.edu)

- Chen, Y. *et al. Nature* 452, 429–435 (2008)
- Edelson, S. & Osherovich, L. *SciBX* 2(16); doi:10.1038/scibx.2009.64

## COMPANIES AND INSTITUTIONS MENTIONED

- Alnylam Pharmaceuticals Inc.** (NASDAQ:ALNY), Cambridge, Mass.  
**Biogen Idec Inc.** (NASDAQ:BIIB), Weston, Mass.  
**Isis Pharmaceuticals Inc.** (NASDAQ:ISIS), Carlsbad, Calif.  
**Merck & Co. Inc.** (NYSE:MRK), Whitehouse Station, N.J.  
**Mount Sinai School of Medicine**, New York, N.Y.  
**Regulus Therapeutics Inc.**, San Diego, Calif.  
**Sage Bionetworks**, Seattle, Wash.  
**Sangamo BioSciences Inc.** (NASDAQ:SGMO), Richmond, Calif.  
**University of Washington**, Seattle, Wash.

or laboratory infrastructure, then that could be a big advance towards a point-of-care solution.”

“The approach is an interesting idea and different from conventional detection methods as it relies on a probe that gets modified by an enzyme produced by *Mtb*,” said Deborah Hung, an assistant professor in the Department of Molecular Biology at **Massachusetts General Hospital** and in the Department of Microbiology and Immunobiology at **Harvard Medical School**. “Other methods to detect the pathogen have typically relied on compounds that bind to certain components from the bacterium.”

### Optimizing TB diagnosis

Global BioDiagnostics president and cofounder Chris Thornton said that in addition to potentially improving the diagnosis of TB in resource-limited settings, the company’s point-of-care test also could help optimize the algorithms used to diagnose TB in countries where more advanced tools and facilities are available.

He gave the example of the semiautomated GeneXpert molecular diagnostics system developed by **Cepheid Inc.**, which is used with Cepheid’s Xpert MTB/RIF cartridges to detect *M. tuberculosis* DNA in clinical samples and to assess the bacterium’s sensitivity to the antibiotic rifampicin within two hours.

“A majority of the time, even in resource-constrained settings, TB diagnostic tests come out negative, so from a throughput and cost perspective, it would be advantageous to use our test to initially screen patients for TB and then follow up on just the positive results with systems such as GeneXpert to confirm the result and test for things like drug susceptibility,” said Thornton.

“We see our technology as being complementary to the GeneXpert system,” Norman told *SciBX*.

Thornton added that Global BioDiagnostics’ point-of-care test also could be useful in monitoring treatment progress. “By requiring live bacteria to trip our test, you can get a quick read on the presence of live bacteria in your samples,” he said.

He noted that such treatment monitoring would be difficult with nucleic acid- and smear microscopy-based techniques. Culture-based techniques could be used but require a few weeks to report a result.

Cepheid did not respond to requests for comment.

### Built to test

Global BioDiagnostics is running additional proof-of-concept studies of the fluorogenic probes using clinical samples and has started developing its reagent system to be used with the point-of-care diagnostic assay.

The company hopes to start field testing the diagnostic toward the end of 2014 and to launch the system in 2015. Global BioDiagnostics expects its partner, the **Foundation for Innovative New Diagnostics** (FIND), to run external clinical trials to validate each step of the diagnostic test development process.

Rao noted that such a system could be a small, single-use, portable device containing the probe with which sputum is collected and transferred into the device, and after a few minutes, read by a small reader or imaged with a cell phone camera. He said the research group also is developing strategies to further improve probe performance to aid the design of next-generation fluorogenic probes.

Validation is going to be the key step forward.

“There have been many promising biomarkers that have never made it

to the clinic, and therefore I would be keen to see if a prototype assay can be developed and validated in rigorous clinical studies,” Pai told *SciBX*. “I would be keen to see if this test will have high specificity in the presence of nontuberculosis mycobacteria. It will also be important to determine if there is sufficient enzyme activity in clinical samples to ensure high sensitivity.”

Hung added that it will also be important to determine the false-positive rate for a test using the probes. She noted that the *M. tuberculosis* burden in unprocessed clinical samples could be very low relative to other  $\beta$ -lactamase-producing bacteria such as *Pneumococcus* and *Pseudomonas*, so even a 1,000-fold increase in selectivity may not be sufficient to prevent other bacteria from activating the probe.

Last March, Global BioDiagnostics signed a co-development agreement with FIND, under which the foundation will provide funding and help with early stage validation studies of the company’s point-of-care TB test.

Norman said it is too early to discuss pricing but told *SciBX* the company is aiming to have the disposables cost well under \$10 per test with only one test required per patient and to utilize a reader that costs below \$1,000.

In June, the **Bill & Melinda Gates Foundation**, the **World Health Organization**’s international drug purchase facility UNITAID and the U.S. government signed an agreement with Cepheid to fund the buy down of the Xpert MTB/RIF test to reduce the cost of each test cartridge to \$9.98 for public sector customers in selected countries. The GeneXpert molecular diagnostic system used to process the test cartridges costs at least \$17,000 and requires about \$450 in annual calibration.

Norman said Global BioDiagnostics’ point-of-care test will not require any specialized lab instrumentation or tools other than a reader, which will facilitate its deployment to resource-limited settings.

Global BioDiagnostics also has received an undisclosed amount of funding from the Texas A&M University system, **Research Valley Funds LLC** and the company’s own management. Norman declined to disclose how far the current funding will take the company.

Stanford and Texas A&M have multiple pending patents covering the fluorogenic probes and the use of bacterial  $\beta$ -lactamases for diagnostic, imaging and therapeutic applications. Global BioDiagnostics has licensed the patents.

Lou, K.-J. *SciBX* 5(36); doi:10.1038/scibx.2012.946  
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**Contact:** Jianghong Rao, Stanford University, Stanford, Calif.  
e-mail: [jrao@stanford.edu](mailto:jrao@stanford.edu)
4. Kong, Y. *et al. Proc. Natl. Acad. Sci. USA* **107**, 12239–12244 (2010)

### COMPANIES AND INSTITUTIONS MENTIONED

**Bill & Melinda Gates Foundation**, Seattle, Wash.  
**Cepheid Inc.** (NASDAQ:CPHD), Sunnyvale, Calif.  
**Foundation for Innovative New Diagnostics**, Geneva, Switzerland  
**Global BioDiagnostics Corp.**, Temple, Texas  
**Harvard Medical School**, Boston, Mass.  
**Massachusetts General Hospital**, Boston, Mass.  
**McGill University**, Montreal, Quebec, Canada  
**Research Valley Funds LLC**, College Station, Texas  
**Stanford University**, Stanford, Calif.  
**Texas A&M Health Science Center**, Bryan, Texas  
**Texas A&M University**, College Station, Texas  
**World Health Organization**, Geneva, Switzerland

## 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
<b>Autoimmune disease</b>				
Autoimmune disease	p38 Mitogen-activated protein kinase (p38 MAPK; MAPK14)	<i>In vitro</i> and rat studies identified a p38 MAPK inhibitor that could help treat autoimmune diseases. p38 MAPK inhibitors reduce inflammatory cytokine production but often have poor oral bioavailability and toxicity caused by off-target activity against the cytochrome P450 (p450) isoenzymes. In cellular assays, tetrahydropyrazolopyrimidine derivatives specifically inhibited p38 MAPK without affecting p450 activity and blocked lipopolysaccharide (LPS)-induced tumor necrosis factor- $\alpha$ (TNF- $\alpha$ ) production. In rat models of acute inflammation and chronic, adjuvant-induced arthritis, a lead member of the series decreased TNF- $\alpha$ production and disease progression compared with vehicle control and showed favorable pharmacokinetics and oral bioavailability. Next steps include testing the inhibitors in additional disease models. At least nine companies have p38 MAPK inhibitors in clinical development for various indications.  <b>SciBX 5(36); doi:10.1038/scibx.2012.947</b> <b>Published online Sept. 13, 2012</b>	Findings unpatented; unavailable for licensing	Asano, T. <i>et al. J. Med. Chem.</i> ; published online Aug. 20, 2012; doi:10.1021/jm3008008 <b>Contact:</b> Toru Asano, Astellas Pharma Inc., Ibaraki, Japan e-mail: <a href="mailto:toru.asano@astellas.com">toru.asano@astellas.com</a>
<b>Cancer</b>				
Acute myelogenous leukemia (AML); acute lymphoblastic leukemia (ALL)	Core-binding factor $\beta$ -subunit (CBFB; CBF $\beta$ ); runt-related transcription factor 1 (RUNX1)	<i>In vitro</i> and mouse studies suggest inhibiting the interaction between RUNX1 and CBF $\beta$ could help treat core-binding factor leukemias, which have mutations that affect <i>RUNX1</i> or <i>CBF<math>\beta</math></i> and account for about 24% of adult AML and 25% of pediatric ALL cases. In an assay that measures the level of interaction between RUNX1 and CBF $\beta$ , a screen of 243,398 compounds identified a benzodiazepine compound that blocked the interaction. In human leukemia cells with CBF $\beta$ fusion proteins, the benzodiazepine induced greater cell death than that seen in leukemia cells lacking CBF $\beta$ fusion proteins. In mice transplanted with the leukemia cells, chemotherapy plus the compound increased survival and decreased leukemic burden compared with chemotherapy alone. Next steps include developing a more potent analog.  <b>SciBX 5(36); doi:10.1038/scibx.2012.948</b> <b>Published online Sept. 13, 2012</b>	Patent application filed; available for licensing	Cunningham, L. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Aug. 21, 2012; doi:10.1073/pnas.1200037109 <b>Contact:</b> Paul Liu, National Institutes of Health, Bethesda, Md. e-mail: <a href="mailto:pliu@mail.nih.gov">pliu@mail.nih.gov</a> <b>Contact:</b> Wei Zheng, same affiliation as above e-mail: <a href="mailto:wzheng@mail.nih.gov">wzheng@mail.nih.gov</a>
Colorectal cancer	R-Spondin 2 (RSPO2); RSPO3	Human genomic studies suggest antagonizing <i>RSPO2</i> and <i>RSPO3</i> gene fusion proteins could be useful for treating colorectal cancer. Sequencing the transcriptomes of 68 colorectal cancer samples identified in-frame gene fusions encoding aberrant forms of <i>RSPO2</i> in 3% of tumors and aberrant forms of <i>RSPO3</i> in 8% of tumors. Human tumors with <i>RSPO2</i> and <i>RSPO3</i> fusion proteins both showed greater oncogenic wingless-type MMTV integration site (WNT) signaling than controls lacking the fusion proteins. Next steps include developing animal models of the tumor-associated gene fusions and testing the effect of targeting these proteins.  <b>SciBX 5(36); doi:10.1038/scibx.2012.949</b> <b>Published online Sept. 13, 2012</b>	Patent and licensing status undisclosed	Seshagiri, S. <i>et al. Nature</i> ; published online Aug. 15, 2012; doi:10.1038/nature11282 <b>Contact:</b> Frederic J. de Sauvage, Genentech Inc., South San Francisco, Calif. e-mail: <a href="mailto:sauvage@gene.com">sauvage@gene.com</a> <b>Contact:</b> Somasekar Seshagiri, same affiliation as above e-mail: <a href="mailto:sekar@gene.com">sekar@gene.com</a>

## This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Neuroendocrine tumors	Multiple endocrine neoplasia I (MEN1; <i>menin</i> )	<p>Mouse studies suggest adenovirus-mediated <i>MEN1</i> gene replacement therapy could help treat pituitary tumors in multiple endocrine neoplasia type 1, an autosomal dominant disorder characterized by neuroendocrine tumors. In mice with a <i>Men1</i> deficiency, intratumoral injection of an adenovirus serotype 5 vector containing the <i>Men1</i> gene decreased tumor cell proliferation compared with injection of a control vector. Next steps include evaluating <i>MEN1</i> gene therapy in additional mammalian models and producing pure preparations of the <i>MEN1</i>-containing vector for use in humans.</p> <p><b>SciBX 5(36); doi:10.1038/scibx.2012.950</b> Published online Sept. 13, 2012</p>	Unpatented; licensing status not applicable	<p>Walls, G.V. <i>et al. Cancer Res.</i>; published online Aug. 21, 2012; doi:10.1158/0008-5472.CAN-12-1821 <b>Contact:</b> Raj V. Thakker, University of Oxford, Oxford, U.K. e-mail: <a href="mailto:rajesh.thakker@ndm.ox.ac.uk">rajesh.thakker@ndm.ox.ac.uk</a></p>
<b>Endocrine/metabolic disease</b>				
Diabetes	Unknown	<p>Rat studies suggest antidiabetic prodrugs engineered to release nitric oxide (NO) could reduce cardiovascular risks associated with treatment. In nonfasted diabetic rats, the prodrugs, synthesized by conjugating NO-releasing chemical groups to the antidiabetic drugs nateglinide or meglitinide, showed anti-hyperglycemic activity comparable to that of the parent drugs. In the rat model, a lead nateglinide-derived prodrug triggered NO-induced vascular relaxation and led to a sustained decrease in blood pressure over three hours compared with no changes for vehicle. Next steps could include testing the prodrugs in other animal models of diabetes. Novo Nordisk A/S and Shionogi &amp; Co. Ltd. market PrandiMet, a combination of repaglinide, a short-acting <math>\beta</math> cell-stimulating meglitinide and metformin, to treat diabetes. Novo Nordisk, Dainippon Sumitomo Pharma Co. Ltd. and Shionogi market NovoNorm, a short-acting <math>\beta</math> cell-stimulating meglitinide, to treat diabetes. Novartis AG and Astellas Pharma Inc. market Starlix nateglinide to treat diabetes.</p> <p><b>SciBX 5(36); doi:10.1038/scibx.2012.951</b> Published online Sept. 13, 2012</p>	Patent and licensing status unavailable	<p>Kaur, J. <i>et al. J. Med. Chem.</i>; published online Aug. 23, 2012; doi:10.1021/jm300997w <b>Contact:</b> Edward E. Knaus, University of Alberta, Edmonton, Alberta, Canada e-mail: <a href="mailto:knaus@ualberta.ca">knaus@ualberta.ca</a></p>
<b>Infectious disease</b>				
Malaria	ATPase Ca <sup>++</sup> transporting plasma membrane 4 (ATP2B4; PMCA4); MARVEL domain containing 3 (MARVELD3)	<p>Genomewide association studies identified two loci associated with risk of severe malaria that could become drug targets. In 1,325 cases of severe malaria and 828 unaffected controls, rs4951074 in <i>ATP2B4</i>, which encodes the main calcium pump on erythrocytes, was associated with reduced risk for the severe form of the disease. In this cohort, the intergenic SNP rs2334880 near <i>MARVELD3</i>, which encodes a tight-junction protein of epithelial and vascular endothelial cells, was associated with increased risk for severe disease. Next steps could include validation of the associations in larger patient cohorts.</p> <p><b>SciBX 5(36); doi:10.1038/scibx.2012.952</b> Published online Sept. 13, 2012</p>	Patent and licensing status unavailable	<p>Timmann, C. <i>et al. Nature</i>; published online Aug. 15, 2012; doi:10.1038/nature11334 <b>Contact:</b> Christian Timmann, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany e-mail: <a href="mailto:timmann@bnitm.de">timmann@bnitm.de</a></p>
<b>Neurology</b>				
Alzheimer's disease (AD)	$\beta$ -Amyloid 42; prion protein (PRNP; PrP; CD230)	<p>Cell culture studies suggest soluble PrP could help treat AD. In a human neuronal cell line, soluble PrP and its N-terminal fragment inhibited the formation of cytotoxic <math>\beta</math>-amyloid 42 assemblies and blocked associated toxicity. Next steps include developing peptides or peptidomimetics that mimic the activity of soluble PrP and evaluating them in models of AD.</p> <p><b>SciBX 5(36); doi:10.1038/scibx.2012.953</b> Published online Sept. 13, 2012</p>	Unpatented; licensing status not applicable; available for partnering and collaboration	<p>Niezanski, K. <i>et al. Sci. Transl. Med.</i>; published online Aug. 22, 2012; doi:10.1074/jbc.C112.400614 <b>Contact:</b> Witold K. Surewicz, Case Western Reserve University, Cleveland, Ohio e-mail: <a href="mailto:witold.surewicz@case.edu">witold.surewicz@case.edu</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Alzheimer's disease (AD)	c-Jun N-terminal kinase 3 (JNK3); MAPK10); $\beta$ -amyloid 42	<i>In vitro</i> and mouse studies suggest JNK3 inhibition could help treat AD. In mouse AD models and patients, JNK3 activity in the frontal cortex was greater than that in healthy controls. In the mouse AD models, a <i>Jnk3</i> deficiency led to lower $\beta$ -amyloid 42 levels in the brain and fewer and smaller plaques than normal <i>Jnk3</i> expression. The <i>Jnk3</i> deficiency also led to increased survival of frontal cortex neurons compared with normal <i>Jnk3</i> expression and long-term retention of fear memories. Planned work includes testing small molecule JNK3 inhibitors in animal models of AD.  <b>SciBX 5(36); doi:10.1038/scibx.2012.954</b> <b>Published online Sept. 13, 2012</b>	Unpatented; available for licensing or partnering	Yoon, S.O. <i>et al. Neuron</i> ; published online Sept. 6, 2012; doi:10.1016/j.neuron.2012.06.024 <b>Contact:</b> Sung Ok Yoon, The Ohio State University, Columbus, Ohio e-mail: <a href="mailto:sung.yoon@osumc.edu">sung.yoon@osumc.edu</a>
Alzheimer's disease (AD)	Mep1n A $\beta$ (MEP1B)	<i>In vitro</i> studies suggest inhibiting MEP1B could help treat AD. In brains from patients with AD, MEP1B levels were greater than those in brains from healthy controls. In human embryonic kidney cells, overexpression of amyloid precursor protein (APP) and MEP1B led to higher $\beta$ -amyloid (A $\beta$ ) levels than normal expression. In the APP- and MEP1B-overexpressing cells, an MEP1B inhibitor decreased A $\beta$ accumulation compared with vehicle. Next steps could include testing MEP1B inhibitors in mouse models of AD.  <b>SciBX 5(36); doi:10.1038/scibx.2012.955</b> <b>Published online Sept. 13, 2012</b>	Patent and licensing status unavailable	Bien, J. <i>et al. J. Biol. Chem.</i> ; published online Aug. 9, 2012; doi:10.1074/jbc.M112.395608 <b>Contact:</b> Claus U. Pietrzik, Johannes Gutenberg University Mainz, Mainz, Germany e-mail: <a href="mailto:pietrzik@uni-mainz.de">pietrzik@uni-mainz.de</a> <b>Contact:</b> Christoph Becker-Pauly, Christian Albrechts University of Kiel, Kiel, Germany e-mail: <a href="mailto:cbeckerpauly@biochem.uni-kiel.de">cbeckerpauly@biochem.uni-kiel.de</a>
Huntington's disease (HD)	Huntingtin (HTT)	<i>In vitro</i> and mouse studies suggest allele-selective single-strand small interfering RNA (ss-siRNA) could help treat HD. In fibroblast cell lines derived from patients with HD, several chemically modified ss-siRNAs selectively silenced mutant but not wild-type <i>HTT</i> alleles at low nanomolar IC <sub>50</sub> values. In mice that heterozygously expressed mutant <i>Htt</i> , intraventricular infusion of a lead ss-siRNA decreased levels of mutant but not wild-type Htt protein in the frontal cortex and other brain areas compared with vehicle. Ongoing work by Isis Pharmaceuticals Inc. includes optimizing the ss-siRNAs against <i>HTT</i> and other undisclosed targets.  <b>SciBX 5(36); doi:10.1038/scibx.2012.956</b> <b>Published online Sept. 13, 2012</b>	Patented by Isis Pharmaceuticals; available for licensing	Yu, D. <i>et al. Cell</i> ; published online Aug. 31, 2012; doi:10.1016/j.cell.2012.08.002 <b>Contact:</b> David R. Corey, The University of Texas Southwestern Medical Center, Dallas, Texas e-mail: <a href="mailto:david.corey@utsouthwestern.edu">david.corey@utsouthwestern.edu</a>
Parkinson's disease (PD); amyotrophic lateral sclerosis (ALS)	Amyloid precursor protein (APP); chromogranin B (CHGB); neuregulin 1 (NRG1); $\alpha$ -synuclein (SNCA)	Mouse and human studies suggest measuring the transport rates of CNS proteins into and out of cerebrospinal fluid (CSF) could help diagnose PD and ALS. In CSF from mouse models for ALS pretreated with heavy water ( <sup>2</sup> H <sub>2</sub> O), rates of appearance and disappearance of <sup>2</sup> H-labeled Chgb, Nrg1 and soluble App were lower than those for healthy <sup>2</sup> H <sub>2</sub> O-pretreated controls. In CSF of <sup>2</sup> H <sub>2</sub> O-pretreated mouse models of PD and in patients with PD, rates of appearance and disappearance of <sup>2</sup> H-labeled CHGB, soluble APP and SNCA were lower than those in healthy <sup>2</sup> H <sub>2</sub> O-pretreated controls. Planned work by KineMed Inc. includes developing the approach to diagnose PD, ALS, Huntington's disease (HD) and Alzheimer's disease (AD). C2N Diagnostics LLC's <sup>13</sup> C-labeling technology is in preclinical development to monitor $\beta$ -amyloid levels in CSF as a measure of treatment response in AD (see <b>This is (a diagnostic) spinal tap, page 4</b> ).  <b>SciBX 5(36); doi:10.1038/scibx.2012.957</b> <b>Published online Sept. 13, 2012</b>	Patented by the University of California, Berkeley; licensed to KineMed; available for licensing or partnering	Fanara, P. <i>et al. J. Clin. Invest.</i> ; published online Aug. 27, 2012; doi:10.1172/JCI64575 <b>Contact:</b> Patrizia Fanara, KineMed Inc., Emeryville, Calif. e-mail: <a href="mailto:pfanara@kinemed.com">pfanara@kinemed.com</a>

## This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Pain	Calcium channel voltage-dependent N type- $\alpha$ 1B subunit (CACNA1B; CaV2.2); collapsin response mediator protein-2 (DPYSL2; CRMP-2)	<i>In vitro</i> and rat studies suggest inhibiting the interaction between CaV2.2 and CRMP-2 could help treat neuropathic pain. CaV2.2 signaling is involved in pain pathways; however, completely blocking the channel affects normal neuron function. In rat dorsal root ganglion neurons, a peptide derived from a Crmp2-binding region of CaV2.2 blocked the Crmp2-CaV2.2 interaction and decreased calcium ion influx and neurotransmitter release compared with a nontargeting peptide. In two rat models of peripheral neuropathy, intraperitoneal administration of the peptide decreased pain behaviors compared with a nontargeting peptide or saline. Next steps include clinical development of a lead molecule. UCB Group markets Vimpat lacosamide, an anticonvulsant that modulates CRMP-2 to slow inactivation of sodium channels, to treat epilepsy and seizures.	Patent application filed covering peptides and their use in preventing neuropathic pain; exclusively licensed to Sophia Therapeutics LLC	Wilson, S.M. <i>et al. J. Biol. Chem.</i> ; published online Aug. 13, 2012; doi:10.1074/jbc.M112.378695 <b>Contact:</b> Rajesh Khanna, Indiana University School of Medicine, Indianapolis, Ind. e-mail: <a href="mailto:khanna5@iupui.edu">khanna5@iupui.edu</a>
Pain	Neuronal pentraxin 1 (NPTX1; NP1)	Rodent studies suggest inhibiting NP1 could help treat neuropathic pain. In a rat model of neuropathic pain, small hairpin RNA against <i>Np1</i> in the rostral ventromedial medulla decreased mechanical allodynia and hyperalgesia compared with control shRNA. In a mouse model of neuropathic pain, <i>Np1</i> knockout decreased mechanical allodynia compared with normal <i>Np1</i> expression. Next steps include replicating the results in additional models of neuropathic pain, identifying the mechanisms underlying the observed effects and developing a method to modulate NP1 function <i>in vivo</i> .	Unpatented; licensing details available from the NIH Office of Technology Transfer	Zapata, A. <i>et al. J. Neurosci.</i> ; published online Sept. 5, 2012; doi:10.1523/JNEUROSCI.2730-12.2012 <b>Contact:</b> Agustin Zapata, National Institute on Drug Abuse Intramural Research Program, Baltimore, Md. e-mail: <a href="mailto:azapata@mail.nih.gov">azapata@mail.nih.gov</a>
Post-traumatic stress disorder	Ryanodine receptor 2 (RyR2)	Mouse studies suggest stabilizing RyR2 could help prevent post-traumatic stress disorder. In mice, an RyR2-stabilizing protein prevented chronic stress-associated learning deficits, memory impairment and anxiety. Ongoing work includes developing a brain-penetrant compound and selecting a molecule for clinical testing.	Patent application filed by Columbia University; licensed to Armgo Pharma Inc.	Liu, X. <i>et al. Cell</i> ; published online Aug. 31, 2012; doi:10.1016/j.cell.2012.06.052 <b>Contact:</b> Andrew R. Marks, Columbia University College of Physicians and Surgeons, New York, N.Y. e-mail: <a href="mailto:arm42@columbia.edu">arm42@columbia.edu</a>
<b>Ophthalmic disease</b>				
Age-related macular degeneration (AMD)	VEGF-A	An <i>in vitro</i> study identified an antagonist of VEGF-A that could be useful for treating AMD. The D-stereoisomer of VEGF-A was synthesized and used to screen for D-protein ligands of VEGF-A. <i>In vitro</i> , the highest-affinity D-protein ligand, D-RFX001, bound the naturally occurring L-stereoisomer of VEGF-A and prevented it from binding the VEGF receptor (VEGFR). Next steps at Reflexion Pharmaceuticals Inc. include optimizing the stability of D-RFX001 and comparing its inhibitory activity with that of Avastin bevacizumab and Lucentis ranibizumab in animal models of AMD. Lucentis, a humanized mAb fragment against VEGF-A, is marketed in the U.S. by Roche's Genentech Inc. unit to treat wet age-related macular degeneration (AMD). Genentech and Chugai Pharmaceutical Co. Ltd. market Avastin, an anti-VEGF mAb, to treat various cancers ( <i>see VEGF reflects on itself, page 1</i> ).	Findings patented; available for licensing from Reflexion Pharmaceuticals	Mandal, K. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Aug. 27, 2012; doi:10.1073/pnas.1210483109 <b>Contact:</b> Sachdev S. Sidhu, University of Toronto, Toronto, Ontario, Canada e-mail: <a href="mailto:sachdev.sidhu@utoronto.ca">sachdev.sidhu@utoronto.ca</a> <b>Contact:</b> Stephen B.H. Kent, The University of Chicago, Chicago, Ill. e-mail: <a href="mailto:skent@uchicago.edu">skent@uchicago.edu</a>
		<b>SciBX 5(36); doi:10.1038/scibx.2012.961</b> Published online Sept. 13, 2012		

## 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
<b>Assays &amp; screens</b>			
Class A $\beta$ -lactamase (blaC)-specific detection of <i>Mycobacterium tuberculosis</i>	Fluorescent probes specific for <i>M. tuberculosis</i> blaC could be useful for detecting <i>M. tuberculosis</i> in clinical samples. Chemically modified cephalosporins that release a fluorophore when bound to blaC were synthesized. <i>In vitro</i> , the probes emitted a fluorescence signal and showed 1,000-fold greater selectivity for blaC over a closely related class A $\beta$ -lactamase produced by other bacterial strains. In unprocessed patient sputum samples, the lead probe detected <i>M. tuberculosis</i> within 10 minutes even when fewer than 10 colony-forming units of bacilli were present. Global BioDiagnostics Corp. is conducting a proof-of-concept study on the probes and has begun development of a diagnostic to detect <i>M. tuberculosis</i> (see <i>Probing for a point-of-care TB test</i> , page 8).	Multiple patents pending; licensed to Global BioDiagnostics	Xie, H. <i>et al. Nat. Chem.</i> ; published online Sept. 2, 2012; doi:10.1038/nchem.1435 <b>Contact:</b> Jianghong Rao, Stanford University, Stanford, Calif. e-mail: <a href="mailto:jrao@stanford.edu">jrao@stanford.edu</a>
	<b>SciBX 5(36); doi:10.1038/scibx.2012.962</b> Published online Sept. 13, 2012		
Human blood metabolite-based timetable	A human blood metabolite-based timetable could help estimate internal body time with just two blood samples. Delivering drugs based on internal body time is associated with improved therapeutic efficacy and reduced side effects, but measuring internal body time requires constant sampling of metabolite levels for more than 24 hours. Blood samples from human subjects were taken every 2 hours over 1.5 days and used to create a reference internal body timetable based on metabolite levels at various time points. In paired human blood samples taken 12 hours apart, the resulting reference metabolite timetable was able to predict internal body time to within 3 hours. Next steps include testing the timetable in additional subjects and developing a strategy to only require a single blood sample.	Patent pending; available for licensing	Kasukawa, T. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Aug. 27, 2012; doi:10.1073/pnas.1207768109 <b>Contact:</b> Hiroki R. Ueda, RIKEN Quantitative Biology Center, Hyogo, Japan e-mail: <a href="mailto:uedah-tyk@umin.ac.jp">uedah-tyk@umin.ac.jp</a> <b>Contact:</b> Tomoyoshi Soga, Keio University, Yamagata, Japan e-mail: <a href="mailto:soga@sfc.keio.ac.jp">soga@sfc.keio.ac.jp</a>
	<b>SciBX 5(36); doi:10.1038/scibx.2012.963</b> Published online Sept. 13, 2012		
Mass spectrometric, antibody-free quantification of proteins in biofluids	A mass spectrometry-based method could be useful for quantifying proteins in biofluids with ELISA-like sensitivity. In the method's first step, microliter volumes of trypsin-digested human serum were fractionated via liquid chromatography-tandem mass spectrometry (LC-MS/MS) into a 96-well plate. In the second step, LC-MS/MS analysis of individual or multiple combined fractions detected peptides corresponding to target proteins, thereby enabling quantification of those proteins at low pg/mL concentrations. In human serum from patients with prostate cancer, the method measured prostate-specific antigen (KLK3; PSA) concentrations with an accuracy comparable to that of ELISA. Ongoing work includes using the technique to quantify undisclosed proteins in human urine.	Unpatented; available for partnering	Shi, T. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Sept. 4, 2012; doi:10.1073/pnas.1204366109 <b>Contact:</b> Wei-Jun Qian, Pacific Northwest National Laboratory, Richland, Wash. e-mail: <a href="mailto:weijun.qian@pnnl.gov">weijun.qian@pnnl.gov</a>
	<b>SciBX 5(36); doi:10.1038/scibx.2012.964</b> Published online Sept. 13, 2012		
<b>Disease models</b>			
Conditional <i>fukutin</i> ( <i>Fktn</i> ) knockout mice as a model for secondary dystroglycanopathies	Conditional <i>Fktn</i> knockout mice could be useful models for secondary dystroglycanopathies, a family of muscular dystrophies caused by mutations in multiple glycosyltransferase genes. In the mice, induced knockout of the glycosyltransferase gene <i>Fktn</i> led to loss of functionally glycosylated dystroglycan 1 (Dag1) within 18 days and development of dystrophic histopathology. In the mice, knocking out <i>Fktn</i> earlier in development led to a more severe disease phenotype compared with knocking out <i>Fktn</i> later in development. Next steps could include using the animals to test therapies for secondary dystroglycanopathies.	Patent and licensing status unavailable	Beedle, A.M. <i>et al. J. Clin. Invest.</i> ; published online Aug. 27, 2012; doi:10.1172/JCI63004 <b>Contact:</b> Kevin P. Campbell, The University of Iowa, Iowa City, Iowa e-mail: <a href="mailto:kevin-campbell@uiowa.edu">kevin-campbell@uiowa.edu</a>
	<b>SciBX 5(36); doi:10.1038/scibx.2012.965</b> Published online Sept. 13, 2012		

## This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
<b>Drug delivery</b>			
Lipid nanoparticle formulation of self-amplifying RNAs for vaccination	Self-amplifying RNA presented in a lipid nanoparticle formulation could be a viable nonviral alternative to viral delivery-based RNA vaccination technologies. In mice and rats, intramuscular injection of a lipid nanoparticle-encapsulated, self-amplifying RNA (LNP/RNA) encoding the RSV F protein had immunogenicity comparable to injection of viral replicon particles. In cotton rats challenged with respiratory syncytial virus (RSV), prophylactic vaccination with the RSV F protein-encoding LNP/RNA provided protection comparable to that of vaccination with viral replicon particles. Ongoing studies include testing the LNP/RNA platform in larger animals.  <b>SciBX 5(36); doi:10.1038/scibx.2012.966</b> <b>Published online Sept. 13, 2012</b>	Patent applications filed; unlicensed	Geall, A.J. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Aug. 20, 2012; doi:10.1073/pnas.1209367109 <b>Contact:</b> Andrew J. Geall, Novartis Vaccines and Diagnostics, Cambridge, Mass. e-mail: <a href="mailto:andrew.geall@novartis.com">andrew.geall@novartis.com</a>
Polyethylene glycol (PEG) coating to improve delivery of drug-loaded nanoparticles across the blood brain barrier (BBB)	Densely coating drug-loaded nanoparticles with PEG could help improve their delivery to the brain. Past studies have suggested nanoparticles need to have a diameter of 64 nm or less to transit the brain's extracellular spaces. In human and rat brain tissue samples, PEG-coated nanoparticles of up to 114 nm diameters diffused into tissues, whereas nanoparticles with carboxyl coatings did not. In rat brain tissue, PEG-coated nanoparticles loaded with paclitaxel diffused rapidly throughout tissues, whereas uncoated drug-loaded nanoparticles did not. Next steps could include using the coating approach to evaluate the delivery of other drugs across the BBB.  <b>SciBX 5(36); doi:10.1038/scibx.2012.967</b> <b>Published online Sept. 13, 2012</b>	Patent pending; available for licensing	Nance, E.A. <i>et al. Sci. Transl. Med.</i> ; published online Aug. 29, 2012; doi:10.1126/scitranslmed.3003594 <b>Contact:</b> Justin Hanes, The Johns Hopkins University, Baltimore, Md. e-mail: <a href="mailto:hanes@jhu.edu">hanes@jhu.edu</a>
<b>Drug platforms</b>			
High throughput flow cytometry for small molecule screening	High throughput, multiplexed flow cytometry could provide a way to rapidly screen small molecules in immune cells. Samples of human peripheral blood mononuclear cells (PBMCs) were labeled with different combinations of 7 unique lanthanide isotopes and arrayed in a 96-well plate. Multiplexed flow cytometry was then used to measure the response of PBMC intracellular signaling to 27 kinase inhibitors under a variety of conditions. The method generated IC <sub>50</sub> values and cellular phosphorylation levels associated with each inhibitor as well as intracellular signaling networks activated by the inhibitors. Next steps include using the method to screen for compounds that modulate epigenetic and metabolic pathways in cancer cells. The method used in the paper is marketed by DVS Sciences Inc. under the name CyTOF.  <b>SciBX 5(36); doi:10.1038/scibx.2012.968</b> <b>Published online Sept. 13, 2012</b>	Flow cytometry method covered by patents owned by DVS Sciences; licensing status undisclosed	Bodenmiller, B. <i>et al. Nat. Biotechnol.</i> ; published online Aug. 19, 2012; doi:10.1038/nbt.2317 <b>Contact:</b> Gary P. Nolan, Stanford University School of Medicine, Palo Alto, Calif. e-mail: <a href="mailto:gnolan@stanford.edu">gnolan@stanford.edu</a>
<b>Markers</b>			
Map of disease gene regulation by noncoding DNA	A genomic study identified noncoding DNA sequences that regulate the expression of disease-related genes and might provide targets that affect disease progression. In a panel of 349 healthy cell and tissue samples, 419 chromosomal sites identified as having a disease-related effect in prior genomewide association studies also regulated the expression of distant genes. Genes regulated by the variants included known disease-associated transcriptional pathways as well as genes that had not previously been linked to disease. Next steps include validating putative disease genes in cell culture and animal models of disease (see <i>Cracking ENCODE</i> , page 6).  <b>SciBX 5(36); doi:10.1038/scibx.2012.969</b> <b>Published online Sept. 13, 2012</b>	Patent pending; available for licensing	Maurano, M.T. <i>et al. Science</i> ; published online Sept. 5, 2012; doi:10.1126/science.1222794 <b>Contact:</b> John A. Stamatoyannopoulos, University of Washington, Seattle, Wash. e-mail: <a href="mailto:jstam@uw.edu">jstam@uw.edu</a>

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