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Engaging drug discovery

By Amy Donner, Senior Editor

Determining whether a compound binds its intended target is a fundamental question in drug discovery, but assays for measuring target engagement *in vivo* can be indirect and inconclusive. To remedy the situation, a Swedish group has modified an *in vitro* assay so that it can be applied to living systems.¹

The team founded **Pelago Bioscience AB** to optimize the assay, which they will provide as a service to aid drug discovery programs at client companies, and to adapt the assay for clinical applications.

Current tools that quantify target engagement *in vitro* include the thermal shift-based assay (TSA), ligand displacement assays, chemoproteomics and tests that look at changes in substrate or product concentrations.

TSAs measure shifts in a protein's thermal stability that occur when bound by a therapeutic. Purified proteins are subjected to gradually increasing temperatures in the presence and absence of a bound ligand and are monitored by fluorescence- or light scattering-based approaches. The extent of temperature shift between the two conditions is proportional to the affinity of the ligand for the target, or the median IC₅₀ value.

Existing *in vivo* assays for quantifying target engagement have multiple shortcomings.

"There is an urgent need for relevant and robust target engagement assays in drug discovery," said Pär Nordlund, a professor of medical biochemistry and biophysics at the **Karolinska Institute** and scientific advisor and cofounder of Pelago. "Current assays can be indirect and often dependent on soft biological readouts. This can introduce uncertainties at many steps along the drug discovery process. In extreme cases, compounds have entered clinical trials and later been shown to not even hit their anticipated targets."

To address these problems, a group led by Nordlund and Daniel Molina developed a cellular thermal shift assay (CETSA) for assessing target engagement *in vivo*. Molina is senior lab manager at Karolinska and CSO at Pelago.

To carry out a CETSA, multiple aliquots of a soluble protein fraction in cell lysates are heated to different temperatures, cooled down and centrifuged to remove any proteins that fall out of solution. The amount of target proteins remaining in solution is quantified by western blotting.

Plotting the quantity of soluble target versus the temperature profile yields a melting curve. As in the *in vitro* version of the assay,

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SciBX is produced by BioCentury Publications, Inc. and Nature Publishing Group Joint Steering Committee: Karen Bernstein, Ph.D., Chairman & Editor-in-Chief, BioCentury; David Flores, President & CEO, BioCentury; Bennet Weintraub, Finance Director, BioCentury; Steven Inchoombe, Managing Director, Nature Publishing Group; Peter Collins, Ph.D., Publishing Director, NPG; Christoph Hesselmann, Ph.D., Chief Financial Officer, NPG.

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the addition of a therapeutic that stabilizes the target causes the thermal curve to shift.

In proof-of-concept experiments, known binders of four therapeutic target proteins—cyclin dependent kinase 2 (CDK2), CDK6, BRAF and methionine aminopeptidase 2 (MetAP2)—triggered shifts in thermal stability. The team tested the approach in lysates, cells and tissues from mice that received the small molecules.

The researchers also used a CETSA with poly(ADP-ribose) polymerase-1 (PARP-1) and showed that olaparib but not iniparib induced a thermal shift in the target.

Olaparib is a PARP-1 inhibitor from **AstraZeneca plc** that is in Phase II testing for cancer. In June, **Sanofi** discontinued development of iniparib after the putative PARP-1 inhibitor failed to meet the primary endpoint of improved overall survival in a Phase III trial. In 2011 and 2012, papers were published showing that iniparib did not bind its target.^{2,3}

The CETSA findings were published in *Science*. The team included scientists from **Nanyang Technological University** and **Linköping University**.

Nordlund sees CETSA as a way to “improve the odds for selecting the right targets and validating them for therapeutic development, and to enable companies to select the optimal ligands as clinical candidates.”

Brian Hubbard noted that the authors’ experiments do not establish that the assay can be used during candidate optimization because they used compounds that already had been optimized. “During optimization, compounds always have off-target effects. If the CETSA reports no thermal shift, you know the compound isn’t binding the target, which is important, but the presence of a thermal shift is not necessarily specific to the target you’re interested in.”

Hubbard is director of the Therapeutics Projects Group at the **Broad Institute of MIT and Harvard** and was formerly senior director of cardiovascular diseases at **Merck & Co. Inc.** and director of cardiovascular and metabolism research at the **Novartis Institutes for BioMedical Research**.

Benjamin Cravatt, professor and chair of chemical physiology at **The Scripps Research Institute**, noted that the assay works only for soluble proteins and will not be useful for membrane proteins, which constitute a sizable portion of therapeutic targets. Protein complexes also could be difficult to use in the assay, he said.

Hubbard was less concerned about the issue of comprehensive coverage. “It is true that there are targets that this assay will simply not work for, but there is no assay that works for everything,” he said.

Thus, he sees the clinical application of CETSA as promising but cautioned that there are multiple remaining hurdles. For example, he said that the assay can reliably report whether or not a drug bound to its target but that it is more difficult to know how much of the target was bound.

Cravatt did say that the assay “is probably going to have some real

“There is an urgent need for relevant and robust target engagement assays in drug discovery.”

**—Pär Nordlund,
Karolinska Institute**

applications because it is easy for people to do. It is much simpler than other kinase assays; no mass spectrometry is needed—just a western blot.”

Stefan Knapp, a professor of structural biology at the **University of Oxford** who has used *in vitro* TSAs,⁴ also thinks CETSA could have some useful applications. CETSA “provide information about drug binding in cells, whereas conventional assays provide information about loss of function of a target. The assay will be very useful for targets for which no specific biomarker exists.”

Ready, CETSA, go

Pelago plans to work with other companies, performing CETSA-based target engagement studies as a service to aid drug discovery efforts. In addition, Pelago is adapting the CETSA for diagnostic and personalized healthcare applications.

“Imagine that large Phase III studies could be conducted and analyzed knowing the exact target engagement status of all enrolled patients. This could help explain poor [responders] and nonresponders,” said Nordlund.

Pelago already has data indicating that the method works with serum samples as well as with biopsies from patient tumors. He has

filed for a patent covering CETSA as a method to measure ligand binding in complex media. Licenses are available.

Donner, A. *SciBX* 6(30); doi:10.1038/scibx.2013.778
Published online Aug. 8, 2013

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PTC readthrough

By Chris Cain, Senior Writer

There is ample evidence that **PTC Therapeutics Inc.**'s lead compound ataluren promotes premature stop codon readthrough *in vivo*, but at least two academic groups have found that the Duchenne muscular dystrophy and cystic fibrosis therapy has no activity in *in vitro* assays used to measure readthrough.^{1,2} Resolving the discrepancy will require a better understanding of ataluren's mechanism of action and could help inform the development of next-generation therapeutics.

Many genetically inherited diseases are caused by nonsense mutations that give rise to premature termination codons (PTCs), which reduce or eliminate the production of full-length, functional protein. These include about 5%–10% of cystic fibrosis (CF) cases and 10%–15% of Duchenne muscular dystrophy (DMD) cases. CF is caused by mutations in the *cystic fibrosis transmembrane conductance regulator* (*CFTR*), and DMD is caused by mutations in *dystrophin* (*DMD*).

Proof of concept for using small molecules to promote PTC readthrough was established by academic studies of the aminoglycoside antibiotic geneticin (G418), which directly binds to ribosomes and alters the fidelity of translation. However, the toxicity of that compound class led researchers to conduct extensive screens to identify nonaminoglycoside compounds with similar effects on translation.

The lead molecule to emerge from these efforts was ataluren (PTC124), a nonaminoglycoside, readthrough-promoting agent developed by PTC Therapeutics.³ The compound has completed a Phase IIb trial in DMD and a Phase III trial in CF. It is under review to treat nonsense-mediated DMD in the EU.

Although it missed the primary functional endpoints in both the Phase IIb and III trials, nonsignificant therapeutic trends were observed, and publications of data from mice or patient samples showed that ataluren significantly increased *dystrophin* and *CFTR* expression compared with controls.^{3,4}

Controversy arose in 2009 when a group led by James Inglese at the **National Human Genome Research Institute** published in the *Proceedings of the National Academy of Sciences* that ataluren can directly bind to firefly luciferase and drive an increase in luminescence in an *in vitro* reporter system.²

Because a screen initially used to identify ataluren relied on increasing firefly luciferase luminescence as a proxy for detecting PTC readthrough,³ this result raised questions about how the compound was selected for further study. In a separate experiment that used a different luciferase protein to measure PTC readthrough, ataluren had no effect.

Inglese, who is head of the laboratory of assay development and screening technology at the **National Center for Advancing Translational Sciences** and adjunct investigator at the National Human Genome Research Institute, declined to comment specifically on ataluren or the new study.

Now, a group at the **University of Dundee** has raised additional questions about whether ataluren promotes readthrough activity *in vitro*.¹

The team used a diverse set of assays designed to measure readthrough *in vitro*, including two different luciferase assays, a β -galactosidase assay and assays measuring collagen expression. The researchers also performed experiments in which the identity of the stop codon was varied to see if nucleotide context played a role in sensitivity to readthrough agents.

In all experiments, geneticin induced dose-dependent stop codon readthrough. Ataluren had no significant effect at any dose in any assay.

Stuart McElroy, the Dundee screening scientist who led the study, told *SciBX* that he did not set out to raise questions about ataluren.

"We originally used ataluren and the aminoglycoside antibiotics as standard molecules to validate the [readthrough] assays we were developing. We felt comfortable that the assays were working and useful because of the positive results with the aminoglycosides," he said. "We could not, however, explain the negative results for ataluren except within the context of the published off-target activity on firefly luciferase. We decided that the best place for the data was in the public domain for the scientific community to judge."

Results were published in *Public Library of Science Biology*.

"Ataluren has been shown to clearly promote readthrough, and the most interesting unresolved question about its mechanism of action is the precise nature of its target."

—PTC Therapeutics Inc.

Context matters

PTC Therapeutics said it disagrees with the general claim that ataluren does not promote readthrough *in vitro*, adding that the use of cDNA templates that lack introns could be responsible for these seemingly contradictory results.

In a statement to *SciBX*, PTC Therapeutics said ataluren has a long history of positive

results in diverse model systems.

"Twelve published studies completely independent of PTC Therapeutics have demonstrated that ataluren promotes readthrough of premature translation termination codons. These results are from a large set of nonsense alleles in diverse experimental systems addressing multiple genetic disorders," the company said.

David Bedwell, professor of microbiology, genetics and cell biology at **The University of Alabama at Birmingham**, agreed. "Nonsense suppression with aminoglycosides is very easy, so just about anyone can get them to suppress nonsense mutations. However, they're not relevant for clinical use," he said. Bedwell is a consultant for PTC Therapeutics and has worked on readthrough compounds including ataluren.

One key difference is that unlike aminoglycosides, the molecular target of ataluren remains unknown, which precludes a precise understanding of how the compound promotes readthrough. "Ataluren has been shown to clearly promote readthrough, and the most interesting unresolved question about its mechanism of action is the precise nature of its target," PTC Therapeutics said.

The company said that because the systems used by McElroy do not contain introns, they may not accurately model how the compound works *in vivo*. "One key element in our studies was the inclusion of an intron in the reporter gene. The McElroy manuscript used cDNAs lacking introns and thus did not take into account the potential impact of the pre-mRNA splicing process on the translation of nonsense-containing mRNAs," the company said. "This difference is important because several studies have shown that mRNAs derived from intronless

precursors or mRNAs otherwise deprived of specific exon-junction complex proteins are markedly deficient in translation activity.”

Olivier Namy, associate scientist at the **Centre National de la Recherche Scientifique (CNRS)** and the **University of Paris-Sud 11**, agreed that this could be important. “One possibility would be that ataluren only acts on

PTCs found in endogenous mRNA and not on PTCs found in reporter systems,” he said. “The main difference between both mRNAs is the presence of introns. This leads to an important difference as endogenous mRNAs are sensitive to nonsense-mediated decay.”

In his own lab, Namy is screening for compounds that promote readthrough.

McElroy agreed that differences could be due to the DNA template used. “We and others have shown that stop codon context, including the downstream nucleotide, results in differences in the fidelity of the stop signal and apparently how much of a readthrough effect geneticin has, although geneticin was active for every sequence,” he said. “There may be more complexity to the story with sequence elements, further downstream or upstream of the stop codon, that are required to observe activity of PTC124 in the *dystrophin* or *CFTR* genes; however, this is just speculation.”

PTC Therapeutics is continuing to work to understand precisely how ataluren acts to promote readthrough. Ataluren is currently in a Phase III open-label trial in patients with DMD, and the company plans to begin a Phase III trial in patients with CF in 2H13.

Inglese said that the controversy illustrates a larger point about the need for redundancy in screening systems. Last year, his team published

“We decided that the best place for the data was in the public domain for the scientific community to judge.”

—**Stuart McElroy,**
University of Dundee

work in *Nature Methods* describing a screening system that integrates readouts from two independent reporter genes.⁵

“One of the broader implications of this work, an area we have explored deeply, points to the need to more fully understand the mechanistic liabilities of technologies forming the basis of high throughput screening assays

and other drug discovery and development processes. In the present work, reporter bias appears to lead to the confounding results, and this is a pervasive screening problem in general,” he said.

Cain, C. *SciBX* 6(30); doi:10.1038/scibx.2013.779
Published online Aug. 8, 2013

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CRACking pancreatitis

By Lev Osherovich, Senior Writer

The handful of companies pursuing CRAC inhibitors for immunological disorders have a new indication to consider—acute pancreatitis. New findings from **Cardiff University**¹ show that the target plays a central role in the painful and potentially life-threatening condition for which there are no FDA-approved, disease-modifying therapies.

Binge drinking can trigger acute pancreatitis, in which excessive release of pancreatic enzymes leads to pain, inflammation and damage to pancreatic tissue.

Treatment focuses on alleviating pain and inflammation or on managing rare metabolic conditions that bring on bouts of the disorder independently of binge drinking. For example, Glybera alipogene tiparvec (AMT-011), a gene therapy from **uniQure B.V.**, is approved in the EU to treat lipoprotein lipase (LPL) deficiency, a hereditary condition that leads to acute, recurring pancreatitis.

The Cardiff team, led by professor and director of biosciences Ole Petersen, now has evidence in cell culture that blocking the CRAC (calcium release-activated calcium channel) can prevent the toxicity and cell death that lead to tissue damage in pancreatitis.

CRAC is a plasma membrane complex that opens up to recharge intracellular calcium when calcium levels in the secretory system fall after release of digestive enzymes (*see* **Figure 1, “CRACkdown on pancreatitis”**).

The complex consists of transmembrane protein 142A (ORAI1; TMEM142A; CRACM1), which forms the channel's pore, and stromal interaction molecule 1 (STIM1), a regulatory subunit associated with the endoplasmic reticulum.

CRAC previously was thought to be expressed primarily in the immune system. Thus, companies including **Synta Pharmaceuticals Corp.** and **CalciMedica Inc.** are developing CRAC inhibitors for immunological disorders.

“To date, CRAC has been detected in numerous different tissues, but since most tissues outside of the immune system possess other channels that can provide intracellular calcium at suitable levels, CRAC has only been [thought to be] essential for the function of lymphocytes,” said Christian Cortis, head of business development at Synta. “These results are novel in that they appear to identify another cell type with significant dependence on CRAC.”

CRACkdown

Petersen said his team had suspected for some time that deranged calcium release played a role in acute pancreatitis,² but it was not clear until now which types of calcium channels were involved.

“We made the original hypothesis in the early 1990s that pancreatitis might be a calcium toxicity disease,” said Petersen. “There's no doubt

that an early step in pancreatitis is calcium channel release from internal stores in response to high concentrations of bile acids or alcohol-related compounds.”

In the new study, Petersen's team used *in vitro* pharmacology and electrophysiology to show that CRAC was involved in normal regulation of calcium in pancreatic cells. GSK-7975A, a CRAC antagonist, blocked the influx of calcium into cultured murine acinar cells depleted of intracellular calcium stores.

GSK-7975A was originally developed by **GlaxoSmithKline plc's** respiratory disease unit as a research tool.

The Cardiff team then used an *in vitro* assay of acinar cell activity in which exposure to fatty acid ethyl esters, a toxic metabolite of fat and alcohol, led to high calcium spikes, excessive enzyme release and cell death.

“We expose cells isolated from mice to a combination of fatty acids and alcohol, a scenario that we think the cells would experience in an alcoholic binge,” said Petersen.

In the fatty acid ethyl ester-treated cells, GSK-7975A prevented calcium spiking, proteolytic enzyme activation and necrosis.

Results were reported in the *Proceedings of the National Academy of Sciences* and were not patented.

CRAC pipeline

Petersen's electrophysiological studies, together with previous genetic studies of hereditary mutations in components of the CRAC complex, suggest the target is likely not required for normal pancreatic secretion and becomes activated only in extreme situations such as alcohol overexposure.

“The absolute dependence of the pancreatic acinar cells on CRAC *in vivo* has yet to be established,” said Cortis. “The absence of digestive problems in children with genetically defective CRACM1 protein may suggest that at least CRACM1 is not essential for normal functioning of the human pancreas.” Petersen's laboratory is undertaking animal studies to establish whether blocking CRAC can prevent acute pancreatitis *in vivo*.

Petersen said current animal models of pancreatitis do not respond to alcohol exposure, making it difficult to develop therapies for alcohol-induced pancreatitis.

“The majority of people in this field have used a hyperstimulation model, in which you treat [rodents] with high doses of the hormones that normally elicit pancreatic secretion,” said Petersen. “The reason that people have stuck with hyperstimulation is that giving alcohol by itself doesn't do very much.”

Petersen suspects that animals will more readily develop pancreatitis when treated with a combination of fatty acids and alcohol, which combine to form fatty acid ethyl esters. “We think it's quite important that there also be a high-fat diet as well as alcohol,” he said.

An open question is whether other calcium channels besides CRAC play a role in acute pancreatitis.

“This paper provides good evidence for a role for CRAC in acute pancreatitis. There is evidence that GSK-7975A works, so it looks and smells like CRAC, but it would be good to test other compounds” that

“This paper provides good evidence for a role for CRAC in acute pancreatitis. There is evidence that GSK-7975A works, so it looks and smells like CRAC, but it would be good to test other compounds [that block other calcium channels].”

—Kenneth Stauderman,
CalciMedica Inc.

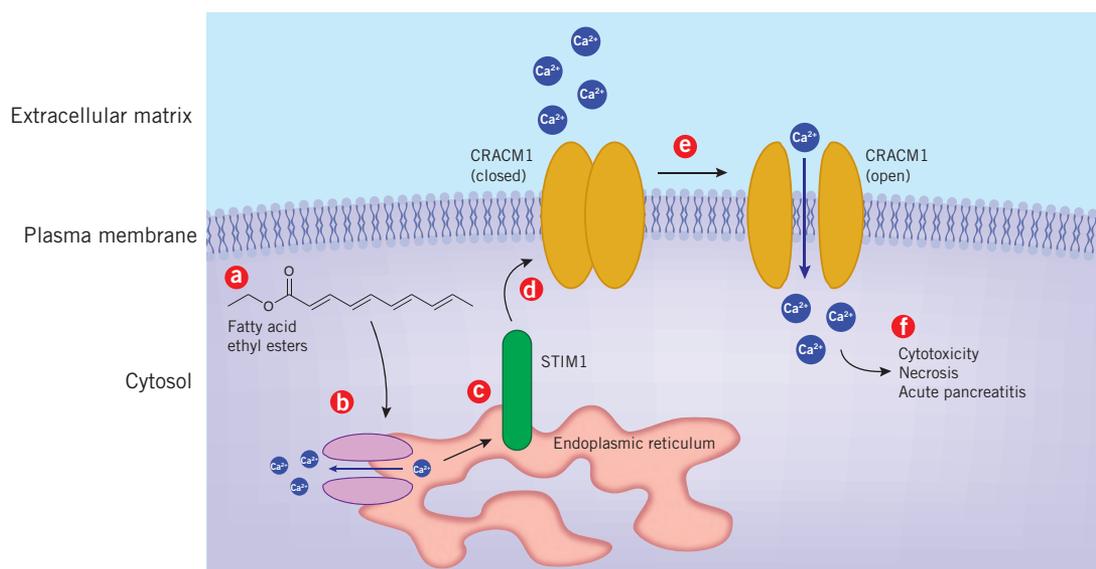


Figure 1. CRACKdown on pancreatitis. Gerasimenko *et al.* have found that blocking calcium release-activated calcium channels (CRACs) could prevent the calcium (Ca^{2+})-mediated cytotoxicity that leads to acute pancreatitis.

CRACs consist of channel subunits such as transmembrane protein 142A (ORAI1; TMEM142A; CRACM1) and calcium-sensing subunits such as stromal interaction molecule 1 (STIM1).

Gerasimenko *et al.* propose that in pancreatic acinar cells, high levels of alcohol lead to formation of fatty acid ethyl esters [a] that cause depletion of calcium stores in the endoplasmic reticulum [b] and activate STIM1 [c]. STIM1 binds to and activates CRACM1 [d], which then opens to allow extracellular calcium to flow into the cytoplasm [e], leading to cytotoxicity, necrosis and pancreatitis [f].

GSK-7975A, a CRACM1 antagonist developed as a research reagent at **GlaxoSmithKline plc** for respiratory indications, prevented acinar cell toxicity caused by fatty acid ethyl esters.

CalciMedica Inc. and **Synta Pharmaceuticals Corp.** have CRACM1 antagonists in preclinical development for autoimmune and inflammatory indications.

block other calcium channels, said Kenneth Stauderman, VP of research at CalciMedica.

What is also missing is evidence that the CRAC components found in rodent acinar cells are present in the human pancreas and are activated in acute pancreatitis.

Stauderman and CalciMedica president and CEO Gonul Velicelebi noted that the specific subunits of CRAC in pancreatic cells may differ from those in immune cells. In human T cells, the principal components of CRAC are CRACM1 and STIM1, but alternative variants of both proteins are known to exist.

“CRACM comes in three flavors, but CRACM1 is apparently the most important for immune cell functions. STIM exists in two forms, but STIM1 is the most relevant in T cells,” said Velicelebi. “We don’t know the selectivity of the GSK compound for CRACM1 versus other variants.”

To establish that CRACM1 and STIM1 are the relevant players in acinar cells, she suggested knocking down either protein and testing whether it leads to an effect similar to that of GSK-7975A.

CalciMedica is developing CRACM1 inhibitors for a range of autoimmune and inflammatory diseases. Last year, the company discontinued its Phase I psoriasis candidate, cm²489, and plans to put a more potent backup compound into Phase I testing next year.

Synta’s preclinical CRACM1 antagonists, STA-12-7525 and STA-12-8336,

were discovered in a now-ended collaboration with **Roche**. Cortis said Synta has full rights to the compounds, which are not undergoing further development and are available for out-licensing.

Cortis, Stauderman and Velicelebi all noted that Petersen’s study suggests their respective companies’ compounds could be tested in pancreatitis but said they did not currently plan to do so.

Osheroich, L. *SciBX* 6(30); doi:10.1038/scibx.2013.780
Published online Aug. 8, 2013

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A new wave of arrhythmia assays

By C. Simone Fishburn, Senior Editor

Cardiotoxicity testing has plagued drug developers for years as the principal preclinical test, the hERG assay, and the primary clinical marker, prolonged QT, are relatively poor predictors of proarrhythmic potential. Now, information about the different ion channels present in the heart and how their interactions with drugs affect the cardiac action potential has opened the door for a wave of new preclinical assays to assess the arrhythmia risk of candidate therapeutics.

The FDA signaled its willingness last month to embrace a new suite of preclinical assays as replacements for both potassium channel Kv11.1 (KCNH2; hERG) testing and thorough QT (tQT) clinical studies. Key stakeholders from the FDA, industry, academia and the investment space met to discuss advances in research and technology that might form the basis of the new recommendations.¹

Since the existing guidelines were introduced in 2005, three particular developments have gained sufficient traction to be included in the new proposals: screening compounds for blockade of multiple cardiac ion channels rather than only for hERG, using cardiomyocytes rather than artificial cellular expression systems such as Chinese hamster ovary (CHO) cells and using *in silico* modeling to simulate how the preclinical findings will affect cardiac function in patients.

The FDA has set a timeline of three years to introduce the new guidelines for preclinical assessment. Although the race is on to select and validate the new assays, the field continues to make breakthroughs that could quickly supersede whatever assays are chosen.

Emerging technologies include patient-derived induced pluripotent stem (iPS) cells that give rise to organ cultures and improved *in silico* simulations with more sophisticated predictive algorithms.

Both sets of advances could ultimately lead to personalized cardiotoxicity testing suited to specific patient subpopulations.

Celling points

Perhaps the most active area in preclinical proarrhythmia research is the creation of improved cell-based systems for testing how candidate compounds affect cardiac function.

Currently, small molecules are routinely tested during preclinical safety studies for binding to hERG. hERG assays typically involve radioligand binding studies or patch clamp recordings on hERG channels expressed either in CHO or human embryonic kidney 293 (HEK293) cells.

The focus on the hERG channel alone is a vast oversimplification of how drugs may predispose patients to arrhythmias because conductances from multiple channels contribute to the cardiac action potential, and drug effects on any of these channels can alter cardiac function.^{2,3}

The most well-validated research under consideration for the new FDA guidelines involves cardiomyocytes derived from human iPS cells that contain all the endogenous cardiac ion channels and spontaneously beat in culture with action potentials that mimic those of a human.⁴

Although iPS cell-derived cardiomyocytes are in use by several companies, they contain a mixture of cardiac cell types and have not yet been fully optimized.

One goal is to create cells identical to human adult ventricular cells because problems during the ventricular repolarization phase of the cardiac action potential are central to the development of arrhythmias.

However, generating a pure population of ventricular cells that can spontaneously beat in culture is a challenge, as the presence of some atrial and nodal cells appears to be necessary for continued generation of the ventricular action potential.

According to Chris Parker, VP and chief commercial officer at **Cellular Dynamics International Inc.**, there is some debate in the field as to whether a pure ventricular cell population is optimal or whether it is preferable to have a pan-population of majority ventricular and minority atrial and nodal cells.

Because drugs can also act on nonventricular cells to interrupt heart function, a mixed population may be able to catch other possible liabilities. However, because ventricular cells represent the most likely target for proarrhythmia effects, diluting

that population may reduce the probability of detecting subtle effects on the ventricular action potential.

CDI has developed a commercial supply of iPS cell-derived cardiomyocytes for use in screening candidate compounds for proarrhythmia potential. The cells are adult-like and produce atrial, nodal and ventricular action potentials that are similar to those of adult human cardiomyocytes.

More importantly, according to Parker, the CDI cells have been shown to respond to a set of ion channel-blocking drugs at the same concentrations that produce effects in human clinical trials, and their results translate more closely to the clinic than those from other existing preclinical models.⁵

“You can’t make cardiac cells that just look like cardiomyocytes and act like them. They have to respond like a clinical patient would,” he told *SciBX*.

Joseph Wu, director of the Stanford Cardiovascular Institute and a professor of medicine and radiology at the **Stanford University School of Medicine**, thinks the next frontier in the field is patient-derived iPS cells that will dominate future preclinical proarrhythmia assessments.

The advantage of using patient-derived iPS cells, according to Wu, is that drugs can be tested on cardiomyocytes representing normal and diseased hearts from multiple ethnic backgrounds, thus providing a window into the range of people likely to take a drug. This would give companies a better assessment of the benefit-risk ratio of each therapeutic candidate and could aid selection of compounds to take into the clinic.

“I would want to test the same drug on 1,000 different cell lines and know why my bottom and top 5% of responders and nonresponders behaved that way so that I could figure out what caused that. That is how

“You can’t make cardiac cells that just look like cardiomyocytes and act like them. They have to respond like a clinical patient would.”

—Chris Parker,
Cellular Dynamics International Inc.

I think drug discovery should be done in the future,” said Wu.

Wu is a cofounder of **Stem Cell Theranostics Inc.**, a startup that has a high throughput screening platform for patient-specific cardiomyocytes. The company’s goal is to partner with biotechs to aid preclinical cardiotoxicity safety testing.

Beyond iPSC cell-derived cardiomyocytes, early stage research on 3D organ cultures and heart-on-a-chip technologies are gaining momentum and could represent the next breakthrough in preclinical cardiotoxicity screening.⁶

3D organ cultures contain the different types of myocardial cells in an organized structure and have the advantage of being able to reproduce disease phenotypes that arise from nonventricular cells. Miniaturizing these on a chip could lead to a high throughput screening system that is affordable for smaller biotechs and could render preclinical personalized cardiotoxicity testing routine in the industry.

Mathematics of the heart

After creating the best cell type to screen in preclinical cardiotoxicity testing, the next step is optimizing the analysis of the data to predict arrhythmia risk.

Gary Mirams, a research fellow at the **University of Oxford**, has developed a computational algorithm that models arrhythmia risk based on the way that cardiomyocyte membrane potential is altered by drugs acting on hERG and other cardiac ion channels. As the membrane potential gets closer to the threshold for triggering an action potential, the likelihood of arrhythmia developing is thought to increase.

Because some drugs block multiple channels, their combined effects on the different channels contribute to their effect on the membrane potential.

For example, verapamil is known to block the hERG channel but does not carry a significant proarrhythmia risk. This is most likely due to the fact that it potently inhibits the calcium channel L-type and causes some blockade of the Nav1.5 (SCN5A) sodium channel.

Thus, its inhibition of the outward potassium flux through the hERG channel would be largely offset by its blockade of the inward calcium and sodium flux, creating a net minimum change for the membrane potential.

Rather than ask “can we predict what happens to a particular ion channel, we ask, ‘Can we predict what happens to a whole heart cell?’” Mirams told *SciBX*.

Mirams’ mathematics-based cardiac electrophysiology model determines the individual channel conductances from IC_{50} values of a compound measured at each channel. It then computes the membrane potential that would result.

Next, the program simulates the overall effect on the cardiac action potential and assesses the likelihood of arrhythmia based on

the large amount of electrophysiological data available from human electrocardiogram studies over the last two decades, linking various action potential measurements to arrhythmia.

Mirams believes incorporating different ethnicities and disease conditions from patient-derived iPSC cells will likely represent the next phase of modeling in this field. This could change the translational strategy in biotechs from assessing the general risk of a compound to assessing the risk of a compound—or even a combination of compounds—in a given patient population, he told *SciBX*.

Mirams’ software is available as open source.

Certara L.P., a healthcare consulting company, recently announced its Cardiac Safety Simulator (CSS) software that incorporates population-based pharmacokinetics to help predict arrhythmia risk.

According to Sebastian Polak, principal scientist at Certara, CSS uses the pharmacokinetics data to account for individual variability in its arrhythmia assessment. It uses parameters from different cardiomyopathies, such as the volume and

area of cardiomyocytes, heart wall thickness, heart rate and plasma concentration, to model how a drug might act in different disease backgrounds.

There is still a long way to go before preclinical data alone could be used to assess proarrhythmia risk. The current momentum for the revised FDA guidelines appears to favor a robust preclinical package combined with rigorous electrocardiogram testing in Phase I trials to replace Phase III tQT studies.

Nevertheless, if progress in the coming years continues at the pace of the last few years, there may be further revisions down the road as the technologies converge to improve cardiotoxicity assessment.

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COMPANIES AND INSTITUTIONS MENTIONED

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“I would want to test the same drug on 1,000 different cell lines and know why my bottom and top 5% of responders and nonresponders behaved that way so that I could figure out what caused that. That is how I think drug discovery should be done in the future.”

—Joseph Wu,
**Stanford University
School of Medicine**

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	Not applicable	Rat studies suggest convection-enhanced delivery (CED) of brain-penetrant nanoparticles loaded with dithiazanine iodide could help treat glioblastoma multiforme (GBM). In a human brain cancer stem cell line, the anthelmintic dye dithiazanine iodide inhibited cellular proliferation with an IC_{50} value of 79 nM. In a rat xenograft model for human GBM, CED of dithiazanine iodide-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles to the brain significantly increased median survival compared with CED of free dithiazanine iodide or empty nanoparticles ($p < 0.005$). Next steps include evaluating the drug-loaded nanoparticles in a clinical trial. SciBX 6(30); doi:10.1038/scibx.2013.782 Published online Aug. 8, 2013	Patent application filed covering use of nanoparticle delivery system in CNS indications; available for licensing from Yale University Contact: Thomas Shrader, Yale University, New Haven, Conn. e-mail: thomas.shrader@yale.edu	Zhou, J. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 1, 2013; doi:10.1073/pnas.1304504110 Contact: W. Mark Saltzman, Yale University, New Haven, Conn. e-mail: mark.saltzman@yale.edu
Breast cancer	Tumor necrosis factor- α (TNF- α)	Mouse studies suggest mAbs that target the transmembrane form of TNF- α could help treat breast cancer. mAbs against TNF- α have been tested in patients with solid tumors but have largely failed to show efficacy. In a mouse xenograft model for human breast cancer, a mAb that specifically targets the transmembrane form of TNF- α decreased tumor volume compared with a control mAb that did not bind TNF- α . Next steps could include evaluating the mAb in additional breast cancer models. SciBX 6(30); doi:10.1038/scibx.2013.783 Published online Aug. 8, 2013	Patent and licensing status unavailable	Yu, M. <i>et al. Cancer Res.</i> ; published online June 21, 2013; doi:10.1158/0008-5472.CAN-12-3946 Contact: Zhuoya Li, Huazhong University of Science & Technology, Wuhan, China e-mail: zhuoyali@mails.tjmu.edu.cn
Cancer	MicroRNA-21 (miR-21)	<i>In vitro</i> and mouse studies suggest inhibiting miR-21 could help treat cancer. High throughput virtual screening identified a molecule that blocked the interaction between pre-miR-21 and dicer 1 ribonuclease type III (DICER1), which processes the pre-miRNA into a mature form. In glioblastoma, breast cancer and gastric cancer cell lines, the miR-21 inhibitor decreased proliferation, migration and invasion and increased apoptosis compared with vehicle. In orthotopic mouse models for glioma and breast cancer, the inhibitor decreased tumor growth and metastasis and increased overall survival compared with vehicle. Next steps include developing derivatives and targeting the treatment to cancers. SciBX 6(30); doi:10.1038/scibx.2013.784 Published online Aug. 8, 2013	Patent application filed covering epithelial cancers; unavailable for licensing	Shi, Z. <i>et al. Cancer Res.</i> ; published online June 28, 2013; doi:10.1158/0008-5472.CAN-13-0280 Contact: Chunsheng Kang, Tianjin Medical University General Hospital, Tianjin, China e-mail: kang97061@gmail.com

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	Not applicable	<p>Mouse studies suggest inhibiting the formation of neutrophil extracellular traps (NETs) could help prevent cancer metastasis after surgery. Postoperative infectious complications such as sepsis have been shown to increase the risk of death from metastatic cancer. In a mouse model for surgery-induced sepsis, injection of murine lung carcinoma cells led to greater hepatic metastasis than sham-operated, nonseptic controls. In septic mice injected with the carcinoma cells, reagents that inhibited NET formation prevented the sepsis-associated increase in hepatic metastasis. Next steps could include developing inhibitors of NET formation that have drug-like properties.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.785 Published online Aug. 8, 2013</p>	Patent and licensing status unavailable	<p>Cools-Lartigue, J. <i>et al. J. Clin. Invest.</i>; published online July 1, 2013; doi:10.1172/JCI67484 Contact: Lorenzo Ferri, McGill University, Montreal, Quebec, Canada e-mail: lorenzo.ferri@mcgill.ca</p>
Cancer	Telomeric repeat binding factor 2 (TERF2)	<p>Mouse and patient sample studies suggest inhibiting TERF2 could help treat cancer by promoting the recruitment of NK cells. In immunocompromised mice injected with transformed fibroblasts with <i>Terf2</i> knockdown, depleting NK cells led to increased tumor formation compared with no depletion. In samples from patients with colon cancer, decreased expression of TERF2 correlated with increased NK cell density during cancer progression. Next steps include screening for compounds that specifically prevent the TERF2-mediated inhibition of NK cells.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.786 Published online Aug. 8, 2013</p>	Unpatented; unavailable for licensing	<p>Biroccio, A. <i>et al. Nat. Cell Biol.</i>; published online June 23, 2013; doi:10.1038/ncb2774 Contact: Eric Gilson, Normal Superior School of Lyon, Lyon, France e-mail: eric.gilson@unice.fr Contact: Annamaria Biroccio, same affiliation as above e-mail: biroccio@ifo.it</p>
Colorectal cancer	Aurora kinase A (AURKA; Aurora-A); AURKB (Aurora-B)	<p><i>In vitro</i> and mouse studies suggest furanopyrimidine-based dual AURKA and AURKB inhibitors could help treat cancer. In <i>in vitro</i> assays, the lead furanopyrimidine selectively inhibited AURKA and AURKB at nanomolar IC₅₀ values. In a mouse xenograft model for human colorectal cancer, the lead compound decreased tumor growth compared with vehicle without causing toxicity. Next steps could include testing the lead compound in animal models for other cancers.</p> <p>Takeda Pharmaceutical Co. Ltd.'s alisertib (MLM8237), a second-generation AURKA inhibitor, is in Phase III testing to treat T cell lymphoma and Phase II testing or earlier for other cancers.</p> <p>EntreMed Inc.'s ENMD-2076, an inhibitor of AURKA and multiple tyrosine kinases, is in Phase II testing to treat sarcoma, breast cancer and ovarian cancer and Phase I testing for other cancers.</p> <p>Astex Pharmaceuticals Inc.'s AT9283, a small molecule inhibitor of AURKA, AURKB, Janus kinase-2 (JAK-2), FMS-like tyrosine kinase 3 (FLT3; CD135) and ABL T315I, is in Phase II testing to treat multiple myeloma (MM) and Phase I testing for other cancers.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.787 Published online Aug. 8, 2013</p>	Patent and licensing status unavailable	<p>Shiao, H.-Y. <i>et al. J. Med. Chem.</i>; published online June 28, 2013; doi:10.1021/jm4006059 Contact: Hsing-Pang Hsieh, National Health Research Institutes, Zhunan, Taiwan e-mail: hphsieh@nhri.org.tw</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Leukemia	Tet methylcytosine dioxygenase 1 (TET1)	<p>Studies in patient samples and in mice suggest inhibiting TET1 could help treat mixed lineage leukemia (MLL). In MLL patient samples, TET1 expression was greater than that in samples from normal controls. In a mouse xenograft model for MLL, small hairpin RNA against <i>Tet1</i> delayed leukemia growth and increased survival compared with control shRNA. Next steps include screening for small molecule inhibitors of TET1.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.788 Published online Aug. 8, 2013</p>	Patent and licensing status undisclosed	<p>Huang, H. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online July 1, 2013; doi:10.1073/pnas.1310656110 Contact: Jianjun Chen, The University of Chicago, Chicago, Ill. e-mail: jchen@medicine.bsd.uchicago.edu Contact: Janet D. Rowley, same affiliation as above e-mail: jrowley@bsd.uchicago.edu</p>
Ovarian cancer	Protein tyrosine phosphatase 1B (PTP-1B: PTPN1); insulin-like growth factor-1 receptor (IGF1R; CD221)	<p>Cell culture studies suggest PTP-1B could help treat IGF1R-dependent ovarian cancer. In IGF1R⁺ ovarian carcinoma cell lines, ectopic overexpression of PTP-1B decreased cell migration, invasion, proliferation and anchorage-independent survival compared with normal PTP-1B expression. Next steps could include testing PTP-1B in mouse xenograft models for ovarian cancer.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.789 Published online Aug. 8, 2013</p>	Patent and licensing status unavailable	<p>Fen, G. <i>et al. J. Biol. Chem.</i>; published online June 28, 2013; doi:10.1074/jbc.M113.482737 Contact: Nicholas K. Tonks, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. e-mail: tonks@cshl.edu</p>
Prostate cancer	Adrenergic receptor β_2 (ADRB2); ADRB3; muscarinic acetylcholine receptor M1 (CHRM1; HM1)	<p>Mouse studies suggest inhibiting autonomic nerve development in the tumor microenvironment could help treat and prevent prostate cancer. In mice implanted with bioluminescent human prostate cancer cells, surgical or pharmacological elimination of sympathetic nerves in the prostate or knockout of <i>ADRB2</i> and <i>ADRB3</i> prevented tumor initiation. In the mouse model, inhibition of parasympathetic neuron signaling by genetic knockout or pharmacological antagonism of <i>Chrm1</i> decreased cell invasion and metastasis compared with no inhibition. Next steps include assessing the safety and efficacy of adrenergic and muscarinic inhibitors in the indication.</p> <p>GlaxoSmithKline plc markets the ADRB2 antagonist Coreg carvedilol to treat heart failure, hypertension and myocardial infarction (MI). Pfizer Inc. and Akrimax Pharmaceuticals LLC market the ADRB2 antagonist Inderal LA propranolol to treat hypertension. At least four companies market CHRM1 antagonists to treat incontinence or drooling.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.790 Published online Aug. 8, 2013</p>	Patent application filed; available for licensing	<p>Magnon, C. <i>et al. Science</i>; published online July 12, 2013; doi:10.1126/science.1236361 Contact: Claire Magnon, Albert Einstein College of Medicine of Yeshiva University, Bronx, N.Y. e-mail: clairemagnon1@gmail.com Contact: Paul S. Frenette, same affiliation as above e-mail: psfrenette@einstein.yu.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Prostate cancer	Androgen receptor (AR); clusterin (CLU; APOJ)	<p>Cell culture and mouse studies suggest dual inhibition of AR and CLU can delay progression of castration-resistant prostate cancer (CRPC). Overexpression of CLU is known to confer resistance to AR inhibitors. In AR-responsive human prostate cancer cell lines, combined treatment with the CLU antisense compound OGX-011 (TV-1011) and the anti-AR drug Xtandi enzalutamide synergistically decreased growth and increased apoptosis compared with either treatment alone. In castrated male mice with human prostate cancer cells, the combination showed greater antitumor effects and increased survival compared with enzalutamide alone. Next steps could include combinatorial clinical studies with inhibitors of AR and CLU.</p> <p>Medivation Inc. markets enzalutamide to treat CRPC. At least four other companies market AR antagonists to treat cancer.</p> <p>OGX-011 from OncoGenex Pharmaceuticals Inc., Isis Pharmaceuticals Inc. and Teva Pharmaceutical Industries Ltd. is in Phase III testing to treat non-small cell lung cancer (NSCLC) and prostate cancer.</p> <p>At least two other companies have CLU inhibitors in preclinical development to treat cancer.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.791 Published online Aug. 8, 2013</p>	Patent and licensing status unavailable	<p>Matsumoto, H. <i>et al. Cancer Res.</i>; published online June 20, 2013; doi:10.1158/0008-5472.CAN-13-0359 Contact: Martin Gleave, The University of British Columbia, Vancouver, British Columbia, Canada e-mail: m.gleave@ubc.ca</p>
Solid tumors	VEGF; VEGF receptor 1 (FLT1; VEGFR-1); VEGFR-2 (KDR/Flk-1)	<p>A mouse study suggests prolonged anti-VEGF therapy to treat cancer could compromise endocrine function of the thyroid gland. In healthy mice, antibodies against VEGF and VEGFR-2 decreased vasculature density in the thyroid and other endocrine tissues compared with an antibody against VEGFR-1 or vehicle. Prolonged anti-VEGF treatment also decreased circulating levels of the thyroid hormone thyroxine compared with vehicle.</p> <p>Next steps include determining the functional impact of prolonged anti-VEGF therapy on endocrine tissues in mouse models and assessing endocrine levels in patients receiving anti-VEGF therapies.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.792 Published online Aug. 8, 2013</p>	Patent and licensing status undisclosed	<p>Yang, Y. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online July 1, 2013; doi:10.1073/pnas.1301331110 Contact: Yihai Cao, Karolinska Institute, Stockholm, Sweden e-mail: yihai.cao@ki.se</p>
Cardiovascular disease				
Ischemia/ reperfusion injury	Adenosine A _{2B} receptor (ADORA _{2B})	<p>Patient sample and mouse studies suggest agonizing ADORA_{2B} could help prevent ischemia and reperfusion injury during liver transplant. In liver biopsies obtained from patients undergoing liver transplant, ADORA_{2B} levels were higher after ischemia and reperfusion.</p> <p>In mice undergoing liver ischemia and reperfusion, pretreatment with an ADORA_{2B} agonist decreased liver injury and inflammation compared with vehicle pretreatment. Next steps include identifying alternative ADORA_{2B} agonists for human disease.</p> <p>Gilead Sciences Inc. and Astellas Pharma Inc., market the ADORA_{2A} agonist Lexiscan regadenoson as a cardiovascular imaging agent.</p> <p>Adenosine Therapeutics LLC's ADORA_{2A} agonist, Stedivaze apadenoson, is in Phase III testing as a cardiovascular imaging agent.</p> <p>Swedish Orphan Biovitrum AB's BVT.115959, an ADORA_{2A} agonist, is in Phase II to treat pain.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.793 Published online Aug. 8, 2013</p>	Unpatented; licensing status not applicable	<p>Zimmerman, M.A. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online June 28, 2013; doi:10.1073/pnas.1221733110 Contact: Holger K. Eltzschig, University of Colorado Denver, Aurora, Colo. e-mail: holger.eltzschig@ucdenver.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Gastrointestinal disease				
Colitis	Free fatty acid receptor 2 (FFAR2; GPR43)	<p>Mouse studies suggest short-chain fatty acid supplementation could be useful for treating colitis and other inflammation-associated colon diseases. Colonic T_{reg} cells have previously been shown to control intestinal inflammation by limiting effector T cell proliferation. In germ-free mice, short-chain fatty acid supplementation increased the number of colonic T_{reg} cells compared with no supplementation. In a T cell transfer mouse model for colitis, supplementation with the FFAR2 agonist propionate or a mixture of short-chain fatty acids decreased disease severity and weight loss compared with no supplementation. Next steps could include screening for and evaluating specific FFAR2 agonists in mouse colitis models.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.794 Published online Aug. 8, 2013</p>	Patent and licensing status unavailable	<p>Smith, P.M. <i>et al. Science</i>; published online July 4, 2013; doi:10.1126/science.1241165 Contact: Wendy S. Garrett, Harvard School of Public Health, Boston, Mass. e-mail: wgarrett@hsph.harvard.edu</p>
Infectious disease				
Sepsis	Angiopoietin 2 (ANG2; ANGPT2)	<p>Mouse studies suggest inhibiting ANG2 could help treat sepsis. ANG2 is upregulated during sepsis. In mice, endothelium-specific ANG2 overexpression increased microvascular disturbances, hypotension and dilatory cardiomyopathy compared with normal expression. In a mouse model for sepsis, two ANG2-targeting antibodies attenuated microvascular and cardiac deterioration and decreased mortality compared with a control antibody. Next steps include testing the antibodies in additional mouse models and larger animal models of sepsis.</p> <p>Roche's RG7221, a bispecific mAb targeting VEGF and ANG2, is in Phase I testing to treat solid tumors. Amgen Inc. and Takeda Pharmaceutical Co. Ltd. have trebananib, a recombinant Fc-peptide fusion protein (peptibody) targeting angiopoietins, in Phase III or earlier testing for various cancers.</p> <p>Silence Therapeutics plc has Atu111, a small interfering RNA lipoplex against ANG2, in preclinical development to treat sepsis.</p> <p>At least three other companies have ANG2-targeting compounds in Phase I testing or earlier to treat cancer or acute lung injury.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.795 Published online Aug. 8, 2013</p>	Antibodies patented by Roche for treatment of cancer, vascular disease and retinopathies; licensing status unavailable	<p>Ziegler, T. <i>et al. J. Clin. Invest.</i>; published online July 1, 2013; doi:10.1172/JCI66549 Contact: Christian Kupatt, Klinikum Grosshadern of the Ludwig Maximilians University, Munich, Germany e-mail: christian.kupatt@med.uni-muenchen.de</p>
<i>Staphylococcus</i>	IL-20 receptor-β (IL20R2; IL20RB)	<p>Mouse and cell culture studies suggest inhibiting IL20RB could help treat methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) skin infections. In a mouse model for MRSA skin infection, an anti-IL20RB mAb dosed at the time of infection decreased lesion size compared with a control antibody. In primary human keratinocytes, exposure to MRSA led to greater expression of cytokines that signal via IL20RB than no MRSA exposure. Next steps could include developing small molecule IL20RB inhibitors.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.796 Published online Aug. 8, 2013</p>	Patent and licensing information unavailable	<p>Myles, I. A. <i>et al. Nat. Immunol.</i>; published online June 23, 2013; doi:10.1038/ni.2637 Contact: Sandip K. Datta, National Institute of Allergy and Infectious Diseases, Bethesda, Md. e-mail: dattas@niaid.nih.gov</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Neurology				
Amyotrophic lateral sclerosis (ALS)	Serine/threonine kinase 4 (STK4); <i>superoxide dismutase 1</i> (SOD1)	Human tissue and mouse studies suggest STK4 inhibitors could help treat ALS. In spinal cord motor neurons from patients with sporadic ALS and mutant SOD1 transgenic mouse models for ALS, levels of activated STK4 were higher than those in neurons from healthy individuals and transgenic mice expressing wild-type SOD1. In the mutant SOD1 mouse model, homozygous <i>Stk4</i> deletion slowed disease onset, increased motor neuron viability and neuromuscular function and decreased mortality compared with no deletion. Ongoing work includes identifying small molecule inhibitors of STK4. SciBX 6(30); doi:10.1038/scibx.2013.797 Published online Aug. 8, 2013	Patent application filed; available for licensing	Lee, J.K. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 1, 2013; doi:10.1073/pnas.1300894110 Contact: Eui-Ju Choi, Korea University, Seoul, South Korea e-mail: ejchoi@korea.ac.kr
Huntington's disease (HD)	Huntingtin (HTT); microRNA-196a (miR-196a)	Mouse and cell culture studies suggest miR-196a could help treat HD. In a mouse model for HD, miR-196a overexpression decreased HD-associated motor deficits and tissue pathology in the brain compared with normal miR-196a expression. In induced pluripotent stem (iPS) cells derived from a patient with HD and differentiated into neuronal cells, miR-196a decreased the accumulation of mutant HTT compared with no miR-196a. Next steps include developing a methodology for <i>in vivo</i> delivery of miR-196a. SciBX 6(30); doi:10.1038/scibx.2013.798 Published online Aug. 8, 2013	Unpatented; licensing status not applicable	Cheng, P.-H. <i>et al. Am. J. Hum. Genet.</i> ; published online June 27, 2013; doi:10.1016/j.ajhg.2013.05.025 Contact: Shang-Hsun Yang, National Cheng Kung University, Tainan, Taiwan e-mail: syang@mail.ncku.edu.tw
Nerve damage	Scavenger receptor class B member 2 (SCARB2)	Cell culture and rat studies suggest SCARB2 could help treat nerve damage. In rat primary dorsal root ganglion (DRG) cells, <i>Scarb2</i> overexpression in cocultured fibroblasts increased neurite outgrowth compared with overexpression of a control gene. In a rat model for neuronal injury, injection of <i>Scarb2</i> -overexpressing fibroblasts increased the number of regenerating DRG axons compared with injection of fibroblasts overexpressing a control gene. Next steps could include testing SCARB2 in additional nerve injury models. SciBX 6(30); doi:10.1038/scibx.2013.799 Published online Aug. 8, 2013	Unpatented; licensing status not applicable	Roet, K.C.D. <i>et al. J. Neurosci.</i> ; published online July 3, 2013; doi:10.1523/JNEUROSCI.1002-13.2013 Contact: Kasper C.D. Roet, Netherlands Institute for Neuroscience, Amsterdam, the Netherlands e-mail: kasperroet@gmail.com
Pain	Acid-sensing ion channel-3 (ASIC3; ACCN3)	<i>In vitro</i> and mouse studies suggest a peptide inhibitor of ASIC3 could help treat inflammatory and acid-induced pain. Electrophysiological testing in transgenic <i>Xenopus laevis</i> oocytes expressing human ASIC3 identified a 29-mer sea anemone peptide as a low micromolar inhibitor of the ASIC3 current. In mouse models for thermal hyperalgesia and acid-induced pain, the peptide decreased hypersensitivity to thermal and acidic stimulus compared with vehicle. Next steps include optimizing the peptide to improve its potency. Theralpha S.A.S. and Flamel Technologies S.A. have THA902, a long-acting formulation ASIC3 inhibitor using Medusa technology, in preclinical testing to treat pain. SciBX 6(30); doi:10.1038/scibx.2013.800 Published online Aug. 8, 2013	Patented by the M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences; available for licensing and partnering	Osmakov, D.I. <i>et al. J. Biol. Chem.</i> ; published June 25, 2013; doi:10.1074/jbc.M113.485516 Contact: Yaroslav A. Andreev, M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia e-mail: ay@land.ru

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Ophthalmic disease				
Diabetic retinopathy	Cysteine-rich angiogenic inducer 61 (CYR61; CCN1)	Patient and mouse studies suggest targeting CCN1 and its degradation products could help treat proliferative diabetic retinopathy. In patients with proliferative diabetic retinopathy, vitreal fluid samples showed higher levels of a truncated, two-domain CCN1 variant than samples from nondiabetic individuals. In mouse models for retinopathy, overexpression of the full-length, four-domain CCN1 or a truncated, three-domain variant decreased pathological ischemia-induced neovascularization compared with expression of a control vector, whereas vector-mediated expression of the truncated, two-domain variant increased pathological neovascularization. Next steps include studies in additional patient samples to determine if clinical parameters of diabetic retinopathy correlate with qualitative and quantitative changes in CCN1.	Patent application filed; licensing details available from the SUNY Downstate Medical Center Office of Scientific Affairs and Biotechnology	Choi, J. <i>et al. J. Biol. Chem.</i> ; published online June 24, 2013; doi:10.1074/jbc.M113.475418 Contact: Brahim Chaqour, SUNY Downstate Medical Center, Brooklyn, N.Y. e-mail: bchaqour@downstate.edu
SciBX 6(30); doi:10.1038/scibx.2013.801 Published online Aug. 8, 2013				
Various				
Colorectal cancer; colitis	c-Src tyrosine kinase (CSK)	Mouse studies suggest activating CSK could help treat colitis and colon cancer. In a mouse model for chemical-induced colitis and colon cancer, a diet including the plant flavonoid isorhamnetin resolved colitis and decreased tumor burden and increased survival compared with a control diet. In colorectal cancer cells, isorhamnetin activity was dependent on increased CSK expression and signaling. Next steps could include identifying the molecular target of isorhamnetin and testing the compound in additional colon cancer models.	Patent and licensing status unavailable	Saud, S.M. <i>et al. Cancer Res.</i> ; published online July 1, 2013; doi:10.1158/0008-5472.CAN-13-0525 Contact: Matthew R. Young, National Cancer Institute, Frederick, Md. e-mail: youngma@mail.nih.gov
SciBX 6(30); doi:10.1038/scibx.2013.802 Published online Aug. 8, 2013				

This week in techniques

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

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

Approach	Summary	Licensing status	Publication and contact information
Assays & screens			
Assay for profiling kinase inhibitor–target interaction in cells	<p><i>In vitro</i> and cell culture studies suggest an assay for monitoring kinase inhibitor–sensitive chaperone-kinase interactions could help identify targets of kinase inhibitors in cells. <i>In vitro</i>, luciferase-tagged heat shock protein 90 (Hsp90) directly bound to BCR-ABL tyrosine kinase, producing a luminescent signal in the assay. In this assay, the nonselective BCR-ABL inhibitor Gleevec imatinib decreased both Hsp90 binding to BCR-ABL and luminescent signal compared with no treatment. The assay showed that a panel of inhibitors selectively blocked kinase-chaperone interactions in a manner consistent with known specificities and identified neurotrophic tyrosine kinase receptor 3 (NTRK3; TrkC) as an additional target of Xalkori crizotinib. Next steps include testing Xalkori in models for tumors caused by NTRK3 mutations, including fibrosarcomas.</p> <p>Novartis AG markets Gleevec to treat chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors.</p> <p>Pfizer Inc. markets Xalkori, a dual inhibitor of c-Met receptor tyrosine kinase and anaplastic lymphoma kinase (ALK), to treat non–small cell lung cancer (NSCLC).</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.803 Published online Aug. 8, 2013</p>	Patent pending; available for licensing	<p>Taipale, M. <i>et al. Nat. Biotechnol.</i>; published online June 30, 2013; doi:10.1038/nbt.2620</p> <p>Contact: Susan Lindquist, Whitehead Institute for Biomedical Research, Cambridge, Mass. e-mail: lindquist_admin@wi.mit.edu</p>
Measuring target engagement in samples	<p>An assay to detect ligand binding in cells could be used to assess target engagement during preclinical and clinical drug development. In cell lysates and in mouse cells and tissues, the cellular thermal shift assay (CETSA) was used to monitor small molecule–induced stabilization of their protein targets by measuring shifts in protein thermal melting curves. In samples from mice, a methionine aminopeptidase 2 (MetAP2) inhibitor shifted the thermal melting curve. Next steps include developing CETSA to monitor target engagement during clinical trials to help explain responders and nonresponders and as a diagnostic tool to inform patient-specific drug selection and dosing (<i>see Engaging drug discovery, page 1</i>).</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.804 Published online Aug. 8, 2013</p>	Patented; available for licensing from Pelago Bioscience AB	<p>Molina, D.M. <i>et al. Science</i>; published online July 5, 2013; doi:10.1126/science.1233606</p> <p>Contact: Pär Nordlund, Karolinska Institute, Stockholm, Sweden e-mail: par.nordlund@ki.se</p>
Disease models			
Cellular model for Down syndrome	<p>A cellular model for trisomy 21 Down syndrome could be useful for studying disease pathophysiology and could help guide the development of new therapeutics. In induced pluripotent stem (iPS) cells derived from a patient with Down syndrome, designed zinc finger nucleases were used to insert inducible <i>X inactive specific transcript (XIST)</i> into chromosome 21. In six clones, induced transgene expression resulted in <i>XIST</i> coating the extra chromosome 21 and triggering chromosome inactivation. In the patient-derived iPS cells, neural differentiation of those with induced <i>XIST</i> expression resulted in normal neural rosette formation, whereas differentiation of cells without induced <i>XIST</i> expression resulted in delayed neural rosette formation. Next steps could include characterizing gene expression and signaling pathway differences between neural progenitor cells from the two different neural rosette populations.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.805 Published online Aug. 8, 2013</p>	Patent and licensing status unavailable	<p>Jiang, J. <i>et al. Nature</i>; published online July 17, 2013; doi:10.1038/nature12394</p> <p>Contact: Jeanne B. Lawrence, University of Massachusetts Medical School, Worcester, Mass. e-mail: jeanne.lawrence@umassmed.edu</p>

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Drug platforms			
Crystal structures of Middle East respiratory syndrome coronavirus (MERS-CoV) spike protein	Crystal structures of the MERS-CoV spike protein in complex with its receptor could aid the development of therapeutics and vaccines against the disease. The MERS-CoV spike protein engages with dipeptidyl peptidase-4 (DPP-4; CD26) expressed on host cells. Researchers solved the crystal structures of the MERS-CoV spike protein receptor-binding domain by itself at 2.5 Å resolution and also in complex with DPP-4 at 2.7 Å resolution. The crystal structures showed that the interaction between the receptor-binding domain of the MERS-CoV spike protein and DPP-4 is mediated primarily by hydrophilic amino acid residues and that the spike protein receptor-binding motif is highly variable. Next steps could include using the crystal structures to design compounds that block the spike protein-CD26 interaction. SciBX 6(30); doi:10.1038/scibx.2013.806 Published online Aug. 8, 2013	Patent and licensing status unavailable	Lu, G. <i>et al. Nature</i> ; published online July 7, 2013; doi:10.1038/nature12328 Contact: George F. Gao, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China e-mail: gaof@im.ac.cn
<i>Escherichia coli</i> coculture for making bispecific antibodies	An <i>E. coli</i> coculture method could be used to make bispecific antibodies. Cocultures of two different <i>E. coli</i> cell types expressing an anti-c-Met proto-oncogene (MET; HGFR) half-antibody with a 'hole' modification at the heterodimerization interface or an anti-epidermal growth factor receptor (EGFR) half-antibody with a 'knob' modification at the heterodimerization interface were harvested and lysed. In the lysate, the half-antibodies spontaneously assembled into a bispecific antibody. In EGFR-driven, MET-driven or MET-EGFR co-dependent xenograft mouse models, the purified bispecific antibody decreased tumor volume compared with vehicle. The coculture method was used to generate 27 additional bispecific antibodies. Next steps include optimizing coculture and purification methods to increase antibody titers and identifying new target combinations. SciBX 6(30); doi:10.1038/scibx.2013.807 Published online Aug. 8, 2013	Patent and licensing status undisclosed	Spiess, C. <i>et al. Nat. Biotechnol.</i> ; published online July 7, 2013; doi:10.1038/nbt.2621 Contact: Justin M. Scheer, Genentech Inc., South San Francisco, Calif. e-mail: scheer.justin@gene.com Contact: Christoph Spiess, same affiliation as above e-mail: spiess.christoph@gene.com
Inhibiting recycling pathways to enhance cellular retention of lipid nanoparticle (LNP)-delivered small interfering RNAs	Inhibiting exocytosis of LNP-delivered siRNAs from endosomes could enhance their retention within the cell. In cultured cells, knockout of <i>Niemann-Pick disease type C1 (NPC1)</i> , which encodes a protein important for endosome recycling, increased siRNA accumulation and gene silencing compared with no knockout. In cells, genetic depletion of other endosome recycling targets yielded comparable outcomes. Next steps could include targeting proteins and lipids involved in biogenesis and endosome trafficking to enhance cellular retention of LNP-delivered siRNA. SciBX 6(30); doi:10.1038/scibx.2013.808 Published online Aug. 8, 2013	Patent and licensing status unavailable	Sahay, G. <i>et al. Nat. Biotechnol.</i> ; published online June 23, 2013; doi:10.1038/nbt.2614 Contact: Daniel G. Anderson, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: dgander@mit.edu
Imaging			
Glucose chemical exchange saturation transfer (glucoCEST) to image enhanced glucose uptake in tumor tissues	Mouse studies suggest the glucoCEST imaging method could help diagnose cancer in a noninvasive manner without needing radioactive tracers. GlucoCEST is an MRI method that can detect uptake of unlabeled glucose without radioactive tracers because of the magnetic effect of the transfer of hydroxyl protons from glucose to water during MRI. In two mouse models with subcutaneous colorectal cancer xenografts, glucoCEST detected higher glucose accumulation in tumor tissues than in muscle tissues and differentiated between tumors with varied metabolic profiles. Next steps could include testing the method in additional cancer models. SciBX 6(30); doi:10.1038/scibx.2013.809 Published online Aug. 8, 2013	Patent and licensing status unavailable	Walker-Samuel, S. <i>et al. Nat. Med.</i> ; published online July 7, 2013; doi:10.1038/nm.3252 Contact: Simon Walker-Samuel, University College London, London, U.K. e-mail: simon.walkersamuel@ucl.ac.uk

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Imaging to quantify lipid nanoparticle (LNP)-mediated delivery of small interfering RNAs	<p>Combination of high-resolution light and electron microscopy (EM) could be used to quantify uptake and trafficking of LNP-delivered siRNAs. In cultured cells, quantitative image analysis of cells treated with fluorescently labeled GFP-targeting siRNAs revealed time-dependent siRNA uptake that corresponded with silencing of GFP. In cells, EM and mathematical modeling revealed an escape rate of 1%–2% for gold-labeled siRNAs from endosomes, corresponding to 2,000–4,000 molecules per cell. In cultured cells and in mice, small molecule-mediated inhibition of endosomal maturation did not affect siRNA escape, indicating that siRNAs escape from early and not mature endosomes. Next steps include identifying small molecules that impact cellular uptake of siRNAs.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.810 Published online Aug. 8, 2013</p>	Unpatented; licensing status not applicable	<p>Gilleron, J. <i>et al. Nat. Biotechnol.</i>; published online June 23, 2013; doi:10.1038/nbt.2612 Contact: Marino Zerial, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany e-mail: zerial@mpi-cbg.de</p>
Markers			
Monitoring conformational variants of α -synuclein (SNCA) to classify neurodegenerative diseases	<p>Distinct conformational variants of SNCA could be useful as biomarkers for distinguishing between subtypes of neurodegenerative diseases including Parkinson's disease (PD). In cultured mouse neurons, expression of two different synthetic conformational variants of human SNCA drove distinct patterns of SNCA and microtubule-associated protein-τ (MAPT; TAU; FTDP-17) inclusion formation. In brain tissue samples from five patients with PD and dementia, two showed expression of a single SNCA conformation, whereas the other three showed expression of two SNCA conformations. Next steps include studies to determine why different SNCA variants produce distinct disease pathologies and to understand the significance of such differences.</p> <p>SciBX 6(30); doi:10.1038/scibx.2013.811 Published online Aug. 8, 2013</p>	Patent application filed covering antibodies against the different SNCA variants; unavailable for licensing	<p>Guo, J.L. <i>et al. Cell</i>; published online July 3, 2013; doi:10.1016/j.cell.2013.05.057 Contact: Virginia M.Y. Lee, University of Pennsylvania School of Medicine, Philadelphia, Pa. e-mail: vmylee@upenn.edu</p>

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