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By Tracey Baas, Senior Editor

GlaxoSmithKline plc and its **Tempero Pharmaceuticals Inc.** spinout have identified selective, first-in-class compounds that bind and inhibit the catalytic domain of class IIa histone deacetylases,¹ a not-so-well-understood subset of the target family that has been genetically linked to several diseases.

Both companies initially plan to study the trifluoromethyloxadiazole (TFMO) molecules in autoimmune disease.

There are four distinct classes of histone deacetylases (HDACs), which are classified according to primary structure: class I (HDAC1, HDAC2, HDAC3 and HDAC8); class II, which is broken down into class IIa (HDAC4, HDAC5, HDAC7 and HDAC9) and class IIb (HDAC6 and HDAC10); class III (sirtuin 1 (SIRT1)–SIRT7); and class IV (HDAC11).

Little is known about the specific cellular and biological functions of each class outside of class I. The difficulty in teasing out that information is exacerbated by the fact that few class-specific HDAC inhibitors exist.

At least 23 companies have HDAC inhibitors in clinical trials, mostly for cancer, and the majority are nonselective. For example, the two marketed HDAC inhibitors, **Merck & Co. Inc.**'s Zolinza vorinostat and **Celgene Corp.**'s Istodax romidepsin, inhibit at least four HDAC isoforms. Both are approved for cutaneous T cell lymphomas (CTCLs).

Development of selective class IIa HDACs has been particularly challenging,^{2,3} in part because the targets have the least mechanistic overlap with other HDAC classes. Indeed, histones are not the substrates of class IIa HDACs—their endogenous enzymatic substrates have not been identified, and their deacetylase activity is almost nonexistent.

Instead, the class IIa HDAC catalytic domain is thought to serve more as an acetyllysine reader, which imprints or erases an epigenetic mark without actually cleaving the post-translational modification, and the noncatalytic domain is thought to take part in multiprotein complexes that can act as transcriptional repressors.

Typically these multiprotein complexes include a class I HDAC to provide deacetylase activity.

Despite the opacity surrounding the function of class IIa HDACs, there have been genetic associations with alopecia, Huntington's disease (HD), glucose homeostasis, muscular dystrophies, autoimmunity and ischemic stroke. Whether the genetic associations are causative or correlative has not been determined, and the dearth of class IIa HDAC inhibitors for use as investigational probes has kept the enzymes poorly validated as targets of human disease.

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The Temporo-GSK team started with recombinant HDAC9 and a synthetic trifluoroacetyllysine analog as a substrate and screened about 2 million compounds from GSK's diversity collection. Of the 93 hits with an IC_{50} value less than 10 μ M, 43 contained the chemical moiety TFMO. Moreover, three of the four most potent hits contained TFMO.

To determine selectivity, the researchers tested the top hit, dubbed TMP269, in HDAC1–11 inhibition assays. In both recombinant enzyme and whole-cell inhibition assays, the small molecule showed preferential inhibition of only class IIa enzymes. The compound did not lead to cytotoxicity in healthy cells, a common side effect of nonselective HDAC inhibitors, at concentrations up to 10 μ M.

The team then worked backward to determine how the TFMO-containing molecules achieved preferential inhibition of class IIa HDACs. The first step was getting a crystal structure of TMP269 bound to HDAC7, which showed that the compound bound directly to the catalytic active site zinc and took on a U-shaped conformation.

Most HDAC inhibitor metal-binding groups, such as hydroxamates, show strong metal-chelating properties.

Based on other reported heterocycle–metal ion interactions, the authors were surprised that the oxygen of the TFMO—not the nitrogen—was closest to the zinc and, together with one of the fluorine atoms, established the metal binding.

Because the catalytic pocket of class IIa HDACs is larger than those of class I and IIb enzymes, the researchers hypothesized that the compound was excluded from binding the zinc of other HDACs because the other classes could not accommodate the large size of TFMO as a metal-binding group.

Indeed, when TFMO was replaced with the smaller metal-chelating hydroxamate, the compound series lost their class IIa HDAC selectivity.

Together, these results indicate that the U-shape conformation and the bulky but weak-binding TFMO group provided class IIa selectivity.

In stimulated human peripheral blood mononuclear cells, one of the lead hits, TMP195, led to gene expression changes in a subset of monocytes but not in T cells or B cells. These gene expression profiles are the first clear identification of transcriptional readouts that show endogenous class IIa HDACs use a catalytic or acetyllysine reader function to modulate monocyte responses to nonspecific stimulation.

Results were published in *Nature Chemical Biology*.

“Having these types of compounds available may help to unveil novel biology that has not been able to be investigated before,” said Christian Steinkühler, scientific director of the **Immaculate's Dermatology Institute** research hospital and treatment center and cofounder and CEO of oncology company **Exiris**.

He was project leader of the Merck team that originally identified trifluoroacetyllysine as the first synthetic *in vitro* substrate of class IIa HDACs.

“Taking this forward by using genomewide acetylproteomics might be most interesting and relevant here. The compounds in the present study are powerful tools for broad scientific exploration, comprising a major advance.”

—James Bradner,
Dana-Farber Cancer Institute

Therapeutic potential

Based on the gene-transcription profiles, Tempero and GSK think their data point toward a role for the class IIa HDACs in macrophage differentiation.

“We first selected to work with immune cells because published work with Hdac9-deficient mice revealed that they were protected in a model of T cell–driven colitis. With these new tool compounds in hand, we are interested to understand their therapeutic potential in autoimmune disease,” said Michael Nolan, principal scientist of biology at Tempero and coleader of the Tempero and GSK team.

“Our far-reaching goals are to further our understanding of class IIa HDACs as therapeutic targets and also to elucidate their molecular activity,” added Nolan. “Our current data demonstrate that occupying the acetyllysine binding site in class IIa HDACs has distinct and measurable consequences in cell biology, providing evidence that the catalytic domains have an endogenous function.”

Timothy McKinsey, associate professor and associate division head for translational research at the **University of Colorado Denver School of Medicine**, wanted to see more details on what happens when class IIa HDACs are blocked. “Even with the compounds in hand, determining an appropriate readout is challenging for measuring the function and inhibition of class IIa HDAC enzymes,” he said. “For other classes, people can rely on monitoring histone acetylation, but not for class IIa enzymes, which lack histone deacetylase activity. These novel compounds should enable discovery of class IIa HDAC substrates.”

“Indeed, taking this forward by using genomewide acetylproteomics might be most interesting and relevant here,” said James Bradner. “The compounds in the present study are powerful tools for broad scientific exploration, comprising a major advance.”

Bradner is an investigator in the Department of Medical Oncology at **Dana-Farber Cancer Institute** and an assistant professor in the Department of Medicine at **Harvard Medical School**. He also is a founder of epigenetics companies **Acetylon Pharmaceuticals Inc.** and **Tensha Therapeutics Inc.**

Steinkühler cautioned that “although a class IIa-specific HDAC inhibitor is available, each individual target—HDAC4, HDAC5, HDAC7 and HDAC9—will likely have its own contribution to biology. Molecular tool optimization should not yet be over.”

Class act

GSK and Tempero are not the only companies pursuing class IIa HDAC inhibitors.

Merck identified a selective HDAC4 inhibitor in 2008, which contained a 2-trifluoroacetylthiophene oxadiazole moiety,⁴ but

“Even with the compounds in hand, determining an appropriate readout is challenging for measuring the function and inhibition of class IIa HDAC enzymes. For other classes, people can rely on monitoring histone acetylation, but not for class IIa enzymes, which lack histone deacetylase activity. These novel compounds should enable discovery of class IIa HDAC substrates.”

—**Timothy McKinsey,**
University of Colorado Denver
School of Medicine

the program was discontinued.

In January, **Novartis AG** filed for a U.S. patent covering TFMO derivatives and their use in treating neurodegeneration, muscle atrophy or metabolic syndrome by way of HDAC4 inhibition.

Meanwhile, **Active Biotech AB** reported last year that its tasquinimod is an allosteric inhibitor of HDAC4.⁵ The compound is in Phase III testing to treat prostate cancer. The intended target of the oral quinoline-3-carboxamide derivative is S100 calcium binding protein A9 (S100A9; calgranulin B; MRP14).

In November 2012, the company showed that the compound interacts with the noncatalytic binding zinc to lock HDAC4 into a conformation that prevents the multiprotein complex formation of HDAC4, nuclear receptor

corepressor 1 (NCOR1) and HDAC3. Blocking this complex inhibits deacetylation of histones and transcription factors such as hypoxia-inducible factor 1 α (HIF1A; HIF1 α). The company said this regulation contributes to tasquinimod’s antiangiogenic and anticancer activity.

The patent status of the molecules described in the *Nature Chemical Biology* paper by Tempero and GSK is undisclosed.

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Glutamine metabolism drives PDAC

By Lauren Martz, Staff Writer

Researchers at the **Dana-Farber Cancer Institute** have identified a glutamine metabolism pathway that is activated by oncogenic K-Ras in pancreatic cancers.¹ Moreover, the team found three different enzyme targets within the pathway that could be blocked to specifically inhibit proliferation of the malignant cells.

Cancer cells have higher metabolic demands than normal cells due to their high levels of growth and proliferation. This increased rate of glycolysis, dubbed the Warburg effect, helps sustain tumor growth.

Recent studies have shown that some oncoproteins, including c-Myc (MYC) and K-Ras, which are known to activate genes involved in cancer cell proliferation, also act as drivers for cancer metabolism.

In a 2012 paper published in *Cell*, a Dana-Farber team led by Alec Kimmelman identified two K-Ras-activated glucose metabolism pathways in pancreatic cancer cells. Inhibiting the pathways helped stop cancer cell proliferation.²

Figure 1. Glutamine metabolism. Cancer cells are dependent on specific metabolic pathways, such as glutamine metabolism, to supply excess energy that sustains cell growth and proliferation and to maintain cellular redox balances.

(I) In a normal glutamine (Gln) metabolic pathway, Gln is transported from the cytoplasm to the mitochondria, where it is converted by glutaminase (GLS) into glutamate (Glu). Glu processing by glutamate dehydrogenase 1 (GLUD1) creates α -ketoglutarate (α -KG), which is used by the tricarboxylic acid (TCA) pathway for aerobic production of energy for the cell.

(II[a]) In pancreatic ductal adenocarcinoma (PDAC) cells, oncogenic K-Ras downregulates GLUD1, which decreases the cancer cells' use of and dependence on the normal Gln pathway.

(II[b]) In an alternative pathway, Glu is converted to aspartic acid (Asp) within the mitochondria by glutamic-oxaloacetic transaminase 2 (GOT2) and then transported to the cytoplasm. Oncogenic K-Ras also upregulates GOT1, a cytoplasmic enzyme that converts Asp to oxaloacetic acid (OAA).

(II[c]) In a downstream series of reactions by malate dehydrogenase 1 (MDH1) and NADP-dependent malic enzyme (ME1), OAA is converted to pyruvate, which reduces NADP⁺ to NADPH. This effect decreases reactive oxygen species within the cancer cells to allow growth and survival.

Inhibition of any of the enzymes in the cancer-upregulated Gln pathway results in increased reactive oxygen species and prevents cancer cell growth.

The *K-Ras* oncogene is expressed in various cancer types, including about 90% of pancreatic cancers. It is associated with poor prognosis and causes resistance to cancer drugs including epidermal growth factor receptor (EGFR) inhibitors. Attempts to target the oncogene directly have not been successful due to the complex biology and interactions of the enzyme's mutant form.

Building on their previous work, Kimmelman and colleagues found that K-Ras activated an anabolic glutamine metabolic pathway specifically in the cancer cells that not only generated energy and building blocks for protein synthesis but also regulated redox homeostasis to allow cancer cell growth.

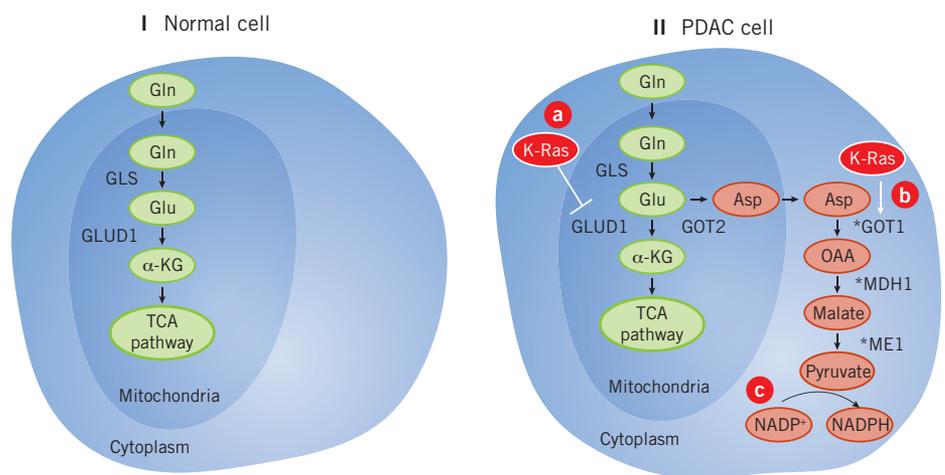
Kimmelman is an assistant professor of radiation oncology at **Harvard Medical School** and Dana-Farber. The paper also included researchers from the **Beth Israel Deaconess Medical Center**, **The University of Texas MD Anderson Cancer Center**, **Weill Cornell Medical College** and **Massachusetts General Hospital**.

Healthy cells employ a glutamine metabolic pathway that converts glutamine-derived glutamate to α -ketoglutarate (α -KG) using the enzyme glutamate dehydrogenase 1

(GLUD1). α -KG is then used to fuel the tricarboxylic acid (TCA) cycle to create energy. In healthy cells, glutamine also can be processed by transaminases in an anabolic pathway that results in both α -KG and the synthesis of nonessential amino acids (NEAAs).

“Even if this strategy proves safe, it might only work for this subset of pancreatic tumors. However, this is a nasty group of tumors, so it would be no small accomplishment.”

—Paul Bingham,
Cornerstone Pharmaceuticals Inc.



The researchers wanted to probe whether cancer cells were dependent on either of these pathways (see Figure 1, “Glutamine metabolism”).

In pancreatic ductal adenocarcinoma (PDAC) cells, glutamine deprivation or inhibition of glutaminase (GLS), the enzyme that converts glutamine to glutamate early in metabolic processing, suppressed cell growth. Addition of NEAAs plus α -KG, but not α -KG alone, restored cell growth in the glutamine-deprived cells.

These findings suggest that the cancer cells depend on the alternative transaminase-dependent glutamine metabolism pathway for proliferation.

For confirmation, the team showed that a nonspecific transaminase inhibitor or a specific inhibitor of the aspartate transaminase glutamic-oxaloacetic transaminase 1 (GOT1) decreased cell growth compared with vehicle. Inhibition of GLUD1 or other transaminases did not affect cell growth.

Genetic knockout studies further showed that GOT1 and the malic enzymes malate dehydrogenase 1 (MDH1) and NADP-dependent malic enzyme (ME1) catalyzed a series of downstream reactions in the pathway that ultimately resulted in the conversion of NADP⁺ to NADPH by pyruvate.

The full picture shows that the pathway maintains low levels of oxidative stress and reactive oxygen species (ROS) within tumors. Indeed, knockdown of pathway enzymes increased ROS levels and inhibited proliferation, whereas treatment with an antioxidant restored cell proliferation.

In mice with human PDAC xenografts, expression of small hairpin RNAs targeting GOT1, MDH1 or ME1 suppressed tumor growth, whereas control shRNA or shRNA targeting GLUD1 did not. In healthy human pancreatic ductal cells or diploid fibroblasts, inhibition of the enzymes did not alter cell growth.

Finally, the team drew a connection between oncogenic *K-Ras* expression and the anabolic glutamine metabolism pathway in PDAC. *K-Ras* knockdown in the cells decreased levels of GOT1 and increased levels of GLUD1, suggesting that the oncogene upregulates enzymes involved in the alternative pathway.

Safety and specificity

“Based on our work, GOT1, MDH1 and ME1 are all potential therapeutic targets,” Kimmelman told *SciBX*. The team now plans to develop inhibitors of the new targets.

Paul Bingham, VP of research at **Cornerstone Pharmaceuticals Inc.**, said, “The most important thing to do next is to find out if you can attack the malic enzymes and transaminases safely. They have identified really clear targets, but they are still a long way from showing that you can attack these enzymes in an intact animal and not cause unwanted toxicities.”

He added that the three new enzyme targets upregulated in tumors are wild-type and thus would be present in some quantities in healthy cells.

Cornerstone’s CPI-613, an analog of α -lipoic acid that targets

pyruvate dehydrogenase (PDH) and α -KG dehydrogenase, is in Phase I/II testing with gemcitabine to treat pancreatic cancer. Bingham said the analog targets multiple activities of the cancer mitochondrial metabolic pathway including redox metabolism.

Neil Jones, senior principal scientist at **Cancer Research UK’s Cancer Research Technology Ltd.** commercial arm, cautioned that “historically, perturbation of the glutamine pathway has caused some concerns in terms of potential toxicity, especially in the brain, so it would be important to assess these liabilities as early as possible in development of potential therapies. The fact that normal cell line models were not affected by glutamine pathway modulations goes some way toward addressing this.”

“Future studies could investigate whether alternative K-Ras-driven tumors, such as non-small cell lung cancers, display this altered glutamine metabolism pathway, opening up the opportunity to extend this therapeutic opportunity into alternative disease segments.”

—Susan Critchlow, AstraZeneca plc

According to Susan Critchlow, associate director of innovative medicines and oncology at **AstraZeneca plc**, “Future studies could evaluate whether small molecule inhibitors of the glutamine metabolism pathway inhibit the growth of established xenograft models and [could] confirm the expected metabolomics profile is desired.”

She noted that the validation studies in the paper only used genetic knockdown approaches in small tumors.

AstraZeneca and Cancer Research Technology are identifying cancer metabolism targets and developing therapeutics under a three-year deal. The partners recently extended the deal for two more years through the beginning of 2015.

Critchlow thinks inhibitors of the enzymes identified in the paper could combine well with standard of care for pancreatic cancer, which involves chemotherapy and radiation. The reason, she said, is that enzyme inhibition disrupts the ability of cancer cells to cope with oxidative stress, and standard of care increases intracellular oxidative stress.

She added, “*GOT1* shRNA-mediated knockdown gives rise to a cytostatic effect *in vivo*, suggesting that combination with standard of care may be required to induce tumor regression.”

Kimmelman agreed. “Our results suggest that since this pathway is critical for redox balance, there could be synergy with available therapies that generate reactive oxygen species,” he said.

Beyond the pancreas

The link between K-Ras and the glutamine metabolism pathway could mean that the three new targets are at work in multiple tumor types.

“Future studies could investigate whether alternative K-Ras-driven tumors, such as non-small cell lung cancers, display this altered glutamine metabolism pathway, opening up the opportunity to extend this therapeutic opportunity into alternative disease segments,” said Critchlow.

Jones agreed. “Defining in more detail the potential patient populations and ascertaining the strengths of the K-Ras lineage against these targets and whether they would help other K-Ras-driven tumor types would also help position these targets,” he said.

Bingham was less sanguine about the broad applicability of the findings.

“The metabolic implications of oncogenic *K-Ras* are different in different cell lines. *K-Ras* may not be activating this particular pathway in other tumor types,” he said. “Even if this strategy proves safe, it might only work for this subset of pancreatic tumors. However, this is a nasty group of tumors, so it would be no small accomplishment.”

Kimmelman told *SciBX* that his team has not tested other cancer types. “However, it is possible that since oncogenic *K-Ras* is responsible for regulating expression of key enzymes in this pathway, that other tumors that have *K-Ras* mutations may also rely on it for growth,” he said. “This will need to be studied.”

He said Dana-Farber has filed a patent application covering the work. The IP is available for licensing.

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Proteoglycans on the knee

By **Tim Fulmer**, Senior Writer

Baylor College of Medicine researchers have shown that delivering the gene encoding proteoglycan 4 directly to the knee prevented and treated osteoarthritis in mice.¹ **GeneQuine Biotherapeutics GmbH** has exclusively licensed the findings and is developing a similar gene therapy to treat osteoarthritis in animals and patients.

Proteoglycan 4 (PRG4; HAPO) is a protein secreted by joint cartilage cells that helps synovial fluid dissipate joint strain. Loss-of-function mutations in PRG4 cause early onset osteoarthritis (OA) associated with the rare condition camptodactyly-arthropathy-coxa vara-pericarditis syndrome.² *Prg4* knockout leads to early development of OA in mice.^{3,4}

Based on those prior results, the Baylor team hypothesized that increasing levels of PRG4 in knee cartilage might help protect against or treat OA associated with aging and traumatic injury, the two most common forms of the disease. The team was led by Brendan Lee, professor and chair of molecular and human genetics.

The researchers first generated mice that overexpressed *Prg4* in cartilage cells. At 10 months, the animals had lower levels of markers of cartilage hypertrophy and degradation and overall lower OA severity than their equally aged wild-type counterparts.

The team next subjected the *Prg4*-overexpressing mice to knee cruciate ligament transection to generate a model of post-traumatic OA. The resulting animals showed less overall OA development following injury than similarly injured wild-type controls.

To translate the genetic overexpression findings into a potential therapy, the researchers tested direct delivery of *PRG4* to the knee in mice. Because long-term expression of *Prg4* might be needed to treat a potentially chronic condition like OA and a recombinant protein could have a short half-life in knee tissue, the researchers relied on gene therapy to deliver a gene overexpressing recombinant *Prg4*.

They used a helper-dependent adenoviral vector (HDV) to deliver the gene because HDVs trigger a mild host immune response compared with first-generation adenoviral vectors, which can trigger a potent immune reaction. HDVs may therefore be better for long-term expression of a recombinant protein.⁵

In mice, intra-articular injection of the HDV-*Prg4* gene therapy protected joints from development of OA following injury.

Results were published in *Science Translational Medicine*.

Getting a leg up

The Baylor researchers will next study the HDV-*Prg4* construct in a horse model of OA, corresponding author Lee told *SciBX*.

That model “is much more meaningful than mouse models since equine joint volumes are closer to human ones and clinical parameters such as lameness, pain and joint effusion can be determined,” said coauthor Kilian Guse, a former postdoctoral researcher in Lee’s lab and CEO and cofounder of GeneQuine.

Lee said the Baylor researchers also plan to build on the paper’s findings by using HDV technology to deliver *PRG4* in combination with other proteins that may show efficacy in OA, such as *IL-1 receptor antagonist (IL-1RA)*. “We must approach OA like treating cancers or infectious disease using combinatorial therapy” because the pathogenesis of OA is multifactorial, he said.

In 2011, Guse founded GeneQuine to develop OA therapies. The company is developing the gene therapy approach to treat animals before going into patients. “We will first focus on horses and dogs since regulatory requirements for veterinary drugs are significantly lower and, therefore, development is markedly cheaper and faster,” said Guse.

GeneQuine’s lead gene therapy product for humans, GQ-203, is in preclinical development to treat OA. The construct uses the HDV platform but delivers an undisclosed therapeutic gene distinct from the *Prg4* gene used in the paper, said Guse.

The company thinks gene therapy is ideal for OA because “high levels of therapeutic protein can be achieved in joints over a long period of time after a single injection of vector,” Guse told *SciBX*.

GeneQuine has an exclusive worldwide license from Baylor to HDVs for the delivery of therapeutic genes and is in the process of licensing the *PRG4*-expressing vector from Baylor, said Guse.

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COMPANIES AND INSTITUTIONS MENTIONED

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“High levels of therapeutic protein can be achieved in joints over a long period of time after a single injection of vector.”

—Kilian Guse,
GeneQuine Biotherapeutics GmbH

The mechanical chemist

By Lev Osherovich, Senior Writer

Cyclofluidic Ltd. has published proof-of-concept data showing that its automated microfluidic lead optimization platform can identify new BCR-ABL tyrosine kinase inhibitors.¹ The company's next step is showing that automated hit-to-lead optimization will work with other targets and compound classes.

Cyclofluidic, a joint venture between **Pfizer Inc.** and **UCB Group**, was formed in 2008 to develop and commercialize drug screening technology centered around a microfluidic robot that integrates the four steps of traditional SAR studies into a single automated cycle (see **Figure 1**, "Automated SAR studies"). The biotech also received an undisclosed amount of seed funding from the U.K.'s **Technology Strategy Board**.

"We have integrated the hardware and software to do synthesis, purification, bioassay and analysis," said team leader and CTO Christopher Selway. "We have a very fast cycle time from synthesis to screening. Once you have your biological data, the system is ready to start on the next compound. We can go around this loop every couple of hours, very quickly building up SAR data. What takes a typical CRO six to eight weeks, we can do in a matter of hours."

Although academic and industry researchers have previously developed semiautomated methods for conducting SAR studies, the new platform is the first working demonstration of a fully integrated system for automatic hit-to-lead optimization.

For this proof of concept, Selway's team started with an already well-characterized and biochemically well-behaved compound class—kinase inhibitors.

Selway's team used the Cyclofluidic platform to find extremely potent derivatives of Iclusig ponatinib, a pan-BCR-ABL tyrosine kinase inhibitor that is marketed by **Ariad Pharmaceuticals Inc.** to treat certain refractory leukemias.

Starting with a library of 10 ponatinib-like templates modified to accept any of 27 substituent groups, the team used computational SAR models to predict 90 new compounds that had the potential to inhibit BCR-ABL at least as potently as the parent compound.

The 90 compounds were synthesized, purified and assayed by the microfluidic apparatus. In line with the computational predictions, four of the compounds had subnanomolar IC_{50} values for BCR-ABL, on par with ponatinib's previously reported potency.

Like ponatinib itself, the four optimized leads also were effective against a variety of mutant forms of BCR-ABL that are resistant to other inhibitors such as **Novartis AG's** Gleevec imatinib.

Results were reported in the *Journal of Medicinal Chemistry*.

"In industry, we crank out whole arrays of compounds, so automating the assaying makes sense. They have the assay in line, which is unusual, then they have an algorithm that helps decide what the next series will be," said Derek Lowe, research fellow at **Vertex Pharmaceuticals Inc.**

Lowe said it is not clear how the Cyclofluidic approach would work beyond kinase inhibitors or whether the compounds coming out of the optimization are necessarily better drugs than the starting compounds.

Because there already is a wealth of knowledge about how kinase inhibitors bind to their targets and ponatinib is already a potent BCR-ABL inhibitor, Lowe said the study effectively started with a stacked deck.

"The automation worked especially well because all kinase inhibitors have similar binding modes, so you can extrapolate a little more," said Lowe. "With a lot of other target classes, the binding modes are unknown because you don't have the structure or you can have multiple modes, so you can't predict how those compounds might bind."

Thus, Lowe said the next step for the Cyclofluidic team should be to demonstrate similar results with nonkinase targets.

Beyond the kinase kingdom

Selway said the platform is indeed best suited for finding small molecule inhibitors of soluble enzymes such as kinases. He added that the company is now working on broadening the platform's range of SAR prediction algorithms and synthesis technologies to cover other chemical classes and targets. His team also is expanding the platform's repertoire of assays with the eventual goal of screening compounds in cell culture.

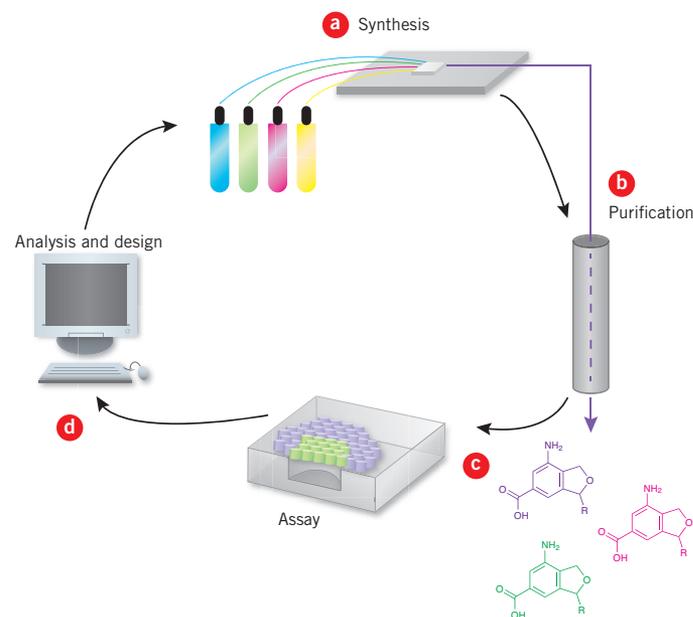


Figure 1. Automated SAR studies. Desai *et al.* at **Cyclofluidic Ltd.** have demonstrated the automated optimization of kinase inhibitors in an integrated synthetic and analytic chemistry platform.

In this scheme, derivatives of a parent compound are synthesized en masse on a microfluidic matrix [a], purified by liquid chromatography and mass spectrometry [b] and then assayed *in vitro* for inhibitory activity against the desired target [c]. A computer program [d] interprets the assay results and suggests further derivatives expected to improve the activity of the best hits, which serve as starting points for the next round of synthesis and screening. The cycle repeats until the operator has obtained SAR-optimized lead compounds with *in vitro* pharmacokinetics superior to those of the parent compound.

“In industry, we crank out whole arrays of compounds, so automating the assaying makes sense. They have the assay in line, which is unusual, then they have an algorithm that helps decide what the next series will be.”

—Derek Lowe,
Vertex Pharmaceuticals Inc.

undisclosed pharma for pilot studies with undisclosed targets.

Lowe cautioned that even if Cyclofluidic can turn around SAR studies faster than human chemists, beating the price of conventional CROs will be challenging. He said the high upfront development cost of customization for different types of targets and assays means the

Selway said that the BCR-ABL compounds are purely proof of concept for the Cyclofluidic platform and will not be advanced further in development.

Cyclofluidic has a fee-for-service business model, and its platform is “open to any company to use on a commercial basis,” said Selway. Since the platform’s commercial launch in mid-2012, the company has signed four deals with

technology is unlikely to be cost effective for now.

Selway countered that the rapid run time and flexibility of the platform could provide savings. He said the company has an 18-month cash runway and hopes to raise additional venture capital to support ongoing development of the platform.

The leads obtained in this study have not been patented.

Osherovich, L. *SciBX* 6(13); doi:10.1038/scibx.2013.304
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- Desai, B. *et al. J. Med. Chem.*; published online Feb. 26, 2013; doi:10.1021/jm400099d

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COMPANIES AND INSTITUTIONS MENTIONED

Ariad Pharmaceuticals Inc. (NASDAQ:ARIA), Cambridge, Mass.

Cyclofluidic Ltd., Welwyn Garden City, U.K.

Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland

Pfizer Inc. (NYSE:PFE), New York, N.Y.

Technology Strategy Board, Swindon, U.K.

UCB Group (Euronext:UCB), Brussels, Belgium

Vertex Pharmaceuticals Inc. (NASDAQ:VRTX), Cambridge, Mass.

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This week in therapeutics

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

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

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Autoimmune disease				
Autoimmune disease	IL-17; serum/ glucocorticoid regulated kinase 1 (SGK1)	Two independent studies suggest inhibiting SGK1 could help treat autoimmune diseases. <i>In vitro</i> , high salt concentrations promoted the differentiation of naïve human CD4 ⁺ cells into T helper type 17 (Th17) cells, induced IL-17 production and increased expression of SGK1. In a mouse model for experimental autoimmune encephalomyelitis (EAE), <i>Sgk1</i> -deficient animals had lower disease incidence and severity than nondeficient controls. Next steps could include testing the therapeutic effect of SGK1 inhibitors in models of Th17 cell-driven autoimmune diseases.	Findings in first study unpatented; licensing status not applicable Patent and licensing status for findings in second study unavailable	Kleinewietfeld, M. <i>et al. Nature</i> ; published online March 6, 2013; doi:10.1038/nature11868 Contact: David A. Hafler, Yale School of Medicine, New Haven, Conn. e-mail: david.hafler@yale.edu Contact: Markus Kleinewietfeld, same affiliation as above e-mail: markus.kleinewietfeld@yale.edu Wu, C. <i>et al. Nature</i> ; published online March 6, 2013; doi:10.1038/nature11984 Contact: Vijay K. Kuchroo, Brigham and Women's Hospital and Harvard Medical School, Boston, Mass. e-mail: vkuchroo@rics.bwh.harvard.edu Contact: Aviv Regev, Broad Institute of MIT and Harvard, Cambridge, Mass. e-mail: aregev@broadinstitute.org
SciBX 6(13); doi:10.1038/scibx.2013.305 Published online April 4, 2013				
Cancer				
Acute promyelocytic leukemia (APL)	PHD finger protein 8 (PHF8)	<i>In vitro</i> and mouse studies suggest activating PHF8 could help restore sensitivity of APL to retinoic acid therapy. In retinoic acid-resistant human APL cells, forced expression of the histone demethylase PHF8 restored sensitivity to all- <i>trans</i> retinoic acid (ATRA), whereas expression of a catalytically inactive mutant PHF8 did not. In mice transplanted with retinoid acid-resistant APL cells that expressed wild-type PHF8, compared with mice given resistant APL cells that expressed catalytically inactive PHF8, ATRA decreased leukemia burden. Next steps include identifying compounds that can increase PHF8 activity or reduce the activity of the counteracting histone methyltransferase.	Unpatented; unavailable for licensing	Arteaga, M.F. <i>et al. Cell</i> ; published online March 18, 2013; doi:10.1016/j.ccr.2013.02.014 Contact: Chi Wai Eric So, King's College London, London, U.K. e-mail: eric.so@kcl.ac.uk
SciBX 6(13); doi:10.1038/scibx.2013.306 Published online April 4, 2013				

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Brain cancer	BRAF; KIAA1549	<p>Cell culture studies suggest second-generation BRAF inhibitors could help treat cancers that harbor the KIAA1549-BRAF fusion protein, which is found in low-grade astrocytomas. In cell lines engineered to express KIAA1549-BRAF, the second-generation BRAF inhibitor PLX PB-3 showed more potent inhibition of the kinase's signaling than the first-generation BRAF inhibitor Zelboraf vemurafenib. In cell lines that expressed KIAA1549-BRAF, PLX PB-3 decreased anchorage-independent growth and cell proliferation rates compared with no treatment. Next steps include developing additional targeting approaches against tumors that are dependent on mutant BRAF and BRAF fusion proteins. Daiichi Sankyo Co. Ltd., Chugai Pharmaceutical Co. Ltd. and Roche market Zelboraf, an oral small molecule inhibitor of oncogenic BRAF V600E, to treat melanoma. The drug is in Phase II testing for thyroid cancer. Daiichi has second-generation BRAF inhibitors in preclinical development. Research-grade vemurafenib and PLX PB-3 were provided by the Plexxikon Inc. unit of Daiichi.</p> <p>SciBX 6(13); doi:10.1038/scibx.2013.307 Published online April 4, 2013</p>	Unpatented; licensing status not applicable	<p>Sievert, A.J. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online March 25, 2013; doi:10.1073/pnas.1219232110 Contact: Adam C. Resnick, The Children's Hospital of Philadelphia, Philadelphia, Pa. e-mail: resnick@email.chop.edu</p>
Breast cancer	Chemokine CXC motif ligand 12 (CXCL12; SDF-1); CXC chemokine receptor 4 (CXCR4; NPY3R)	<p>Mouse studies suggest an oncolytic virus encoding a CXCR4 antagonist could help treat and prevent breast cancer metastasis. An oncolytic vaccinia virus was engineered to encode a fragment of the CXCR4 antagonist CTCE-9908 fused with a murine Fc fragment. In a mouse model for mammary cancer, i.v. treatment with the oncolytic virus decreased tumor volumes compared with treatment using the fusion protein itself or the same virus expressing a control protein. In a mouse model for metastatic mammary cancer, the oncolytic virus decreased lung metastases and increased survival compared with the same virus expressing a control protein. Next steps include evaluating the oncolytic virus therapy in combination with chemotherapy in breast cancer models. Chemokine Therapeutics Corp. previously tested CTCE-9908 in a Phase I/II trial in solid tumors, but the company has ceased operations.</p> <p>SciBX 6(13); doi:10.1038/scibx.2013.308 Published online April 4, 2013</p>	Patent application filed; licensing status undisclosed	<p>Gil, M. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online March 18, 2013; doi:10.1073/pnas.1220580110 Contact: Danuta Kozbor, Roswell Park Cancer Institute, Buffalo, N.Y. e-mail: danuta.kozbor@roswellpark.org</p>
Cancer	Jumonji domain containing 1A (JMJD1A; TSGA)	<p>Mouse and cell culture studies suggest inhibiting JMJD1A could help circumvent resistance to antiangiogenic cancer therapies. In four human cancer cell lines, hypoxic and nutrient-starved conditions led to greater expression of JMJD1A than nonstressed conditions. In two mouse xenograft models for human cancers, small interfering RNA against JMJD1A increased tumor sensitivity to the antiangiogenic drugs Avastin bevacizumab and Sutent sunitinib compared with scrambled siRNA. Next steps could include screening for pharmacological inhibitors of JMJD1A. Roche's Genentech Inc. unit markets Avastin, a humanized mAb against VEGF, to treat colorectal, lung, renal and brain cancers. Pfizer Inc. markets Sutent, a small molecule that inhibits multiple receptor tyrosine kinases including VEGF receptors, to treat GI, pancreatic and renal cancers.</p> <p>SciBX 6(13); doi:10.1038/scibx.2013.309 Published online April 4, 2013</p>	Patent and licensing status unavailable	<p>Osawa, T. <i>et al. Cancer Res.</i>; published online March 14, 2013; doi:10.1158/0008-5472.CAN-12-3231 Contact: Masabumi Shibuya, Tokyo Medical and Dental University, Tokyo, Japan e-mail: shibuya@ims.u-tokyo.ac.jp</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Pancreatic cancer	Glutamic-oxaloacetic transaminase 1 (GOT1); malate dehydrogenase 1 (MDH1); NADP-dependent malic enzyme (ME1)	<i>In vitro</i> and mouse studies suggest inhibiting cancer-specific glutamine metabolism could help treat <i>K-Ras</i> -mutant pancreatic cancers. In pancreatic ductal adenocarcinoma (PDAC) cells, glutamine deprivation or small hairpin RNA against GOT1, MDH1 or ME1, which are enzymes involved in cancer-associated glutamine metabolism pathways driven by oncogenic <i>K-Ras</i> , led to decreased cell proliferation compared with no glutamine deprivation or control shRNA. In a mouse xenograft model for human PDAC, shRNA against glutamine pathway enzymes led to decreased tumor growth compared with a control shRNA. Next steps include identifying pharmacological inhibitors of GOT1, MDH1 or ME1 (see <i>Glutamine metabolism drives PDAC</i> , page 4).	Patent application filed; available for licensing	Son, J. <i>et al. Nat. Med.</i> ; published online March 27, 2013; doi:10.1038/nature12040 Contact: Lewis C. Cantley, Weill Cornell Medical College, New York, N.Y. e-mail: lec2014@med.cornell.edu Contact: Alec C. Kimmelman, Dana-Farber Cancer Institute, Boston, Mass. e-mail: alec_kimmelman@dfci.harvard.edu
Cardiovascular disease				
Cardiovascular disease	Epoxide hydrolase 2 (EPHX2; CEH)	Mouse studies suggest epoxide hydrolase inhibitors could be useful for preventing cardiac fibrosis. In two mouse models for cardiac fibrosis, epoxide hydrolase inhibitors decreased proinflammatory signaling, cardiac hypertrophy and cardiac fibrosis compared with vehicle. Next steps include preclinical development of the compounds with undisclosed industry partners.	Patent pending; available for licensing	Sirish, P. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 14, 2013; doi:10.1073/pnas.1221972110 Contact: Nipavan Chiamvimonvat, University of California, Davis, Calif. e-mail: nchiamvimonvat@ucdavis.edu
Hematology				
Thalassemia; myeloproliferative disorder	Not applicable	Mouse studies suggest disrupting the interaction between macrophages and erythroid cells could help treat conditions associated with disorders in erythropoiesis, such as β -thalassemia and polycythemia vera (PV). Prior studies suggested macrophages can interact with erythroid cells and regulate their proliferation and survival. In mouse models for β -thalassemia and PV, macrophage depletion restored normal erythropoiesis, normalized red blood cell counts and reversed disease pathology, whereas saline did not. Next steps include identifying the molecules responsible for the interaction between erythroid cells and macrophages and developing compounds to target such molecules.	Unpatented; licensing status not applicable	Ramos, P. <i>et al. Nat. Med.</i> ; published online March 17, 2013; doi:10.1038/nm.3126 Contact: Stefano Rivella, Weill Cornell Medical College, New York, N.Y. e-mail: str2010@med.cornell.edu
Infectious disease				
Malaria	Plasmodium cytochrome bc1	Mouse and cell culture studies suggest the 4(1H)-quinolone-3-diarylether ELQ-300 could help treat liver- and blood-stage malaria infections. In a panel of <i>Plasmodium falciparum</i> and <i>P. vivax</i> clinical isolates, including those that were drug resistant, ELQ-300 showed antiplasmodial activity with single-digit nanomolar IC ₅₀ values. In mouse models for blood- and liver-stage <i>P. berghei</i> or <i>P. yoelii</i> infection, oral treatment with ELQ-300 decreased parasitemia and levels of liver-stage parasites compared with vehicle treatment. Next steps could include evaluating ELQ-300 in nonhuman primate models for malaria infection. Medicines for Malaria Venture has ELQ-300 in preclinical development.	Patent applications filed; licensing status unavailable	Nilsen, A. <i>et al. Sci. Transl. Med.</i> ; published online March 20, 2013; doi:10.1126/scitranslmed.3005029 Contact: Michael K. Riscoe, Portland VA Medical Center, Portland, Ore. e-mail: riscoem@ohsu.edu Contact: Roman Manetsch, University of South Florida, Tampa, Fla. e-mail: manetsch@usf.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Viral infection	Dipeptidyl peptidase-4 (DPP-4; CD26)	<p>Cell culture studies identified DPP-4 as the receptor of human coronavirus–Erasmus Medical Center (hCoV-EMC) and suggest that blocking the protein could help treat infection. In cell culture, hCoV-EMC bound and infected cells that expressed DPP-4 but not cells that lacked the protein. In cells expressing DPP-4, polyclonal DPP-4 antiserum blocked hCoV-EMC infection.</p> <p>However, in cell culture, hCoV-EMC infection could not be blocked with the DPP-4 inhibitors Glactiv sitagliptin, Equa vildagliptin or Onglyza saxagliptin, suggesting binding takes place outside of the enzyme's catalytic region. Next steps include generating humanized mAbs that block the interaction between hCoV-EMC and DPP-4.</p> <p>Merck & Co. Inc. and Ono Pharmaceutical Co. Ltd. market Glactiv sitagliptin to treat diabetes.</p> <p>Novartis AG markets Equa vildagliptin to treat diabetes.</p> <p>Bristol-Myers Squibb Co., AstraZeneca plc and Otsuka Pharmaceutical Co. Ltd. market Onglyza saxagliptin to treat diabetes.</p> <p>SciBX 6(13); doi:10.1038/scibx.2013.314 Published online April 4, 2013</p>	Patent application filed; unlicensed	<p>Raj, V.S. <i>et al. Nature</i>; published online March 13, 2013; doi:10.1038/nature12005</p> <p>Contact: Bart L. Haagmans, Erasmus Medical Center, Rotterdam, the Netherlands e-mail: b.haagmans@erasmusmc.nl</p>
Musculoskeletal disease				
Bone repair	Not applicable	<p><i>In vitro</i> and mouse studies suggest depleting terminally differentiated CD8⁺ effector memory T cells could help prevent delayed fracture healing. In patients with delayed fracture healing, peripheral blood and fracture hematoma samples showed higher levels of terminally differentiated CD8⁺ effector memory T cells than samples from patients with normal fracture healing. In an osteogenic differentiation assay, cytokines produced by these CD8⁺ T cells decreased osteogenic differentiation and bone marrow mesenchymal stem cell viability compared with no treatment. In a mouse model for bone fracture, depletion of these CD8⁺ T cells improved fracture healing. Next steps include validation of these CD8⁺ T cells as a biomarker for delayed fracture healing.</p> <p>SciBX 6(13); doi:10.1038/scibx.2013.315 Published online April 4, 2013</p>	Patent application filed for diagnostic and therapeutic applications; available for licensing	<p>Reinke, S. <i>et al. Sci. Transl. Med.</i>; published online March 20, 2013; doi:10.1126/scitranslmed.3004754</p> <p>Contact: Hans-Dieter Volk, Charité–University Hospital Berlin, Berlin, Germany e-mail: hans-dieter.volk@charite.de</p>
Neurology				
Down syndrome	Sorting nexin family member 27 (SNX27)	<p>Patient sample and mouse studies suggest increasing SNX27 expression could help treat Down syndrome. In brain samples from patients with Down syndrome, SNX27 mRNA and protein levels were lower than those in healthy controls. In <i>Snx27</i>^{-/-} mice, learning, memory and synaptic function were impaired. In a mouse model for Down syndrome, hippocampus injection of an adenovirus expressing human SNX27 rescued synaptic and cognitive deficits. Next steps include screening for compounds that activate SNX27 production.</p> <p>SciBX 6(13); doi:10.1038/scibx.2013.316 Published online April 4, 2013</p>	Findings unpatented; unavailable for licensing	<p>Wang, X. <i>et al. Nat. Med.</i>; published online March 24, 2013; doi:10.1038/nm.3117</p> <p>Contact: Huaxi Xu, Xiamen University, Xiamen, China e-mail: hxxu@xmu.edu.cn</p> <p>Contact: Wanjin Hong, Sanford-Burnham Medical Research Institute, La Jolla, Calif. e-mail: xuh@sanfordburnham.org</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Fragile X syndrome	Fragile X mental retardation 1 (FMR1); p21 protein (Cdc42 Rac)-activated kinase (PAK)	<p>Mouse studies suggest the small molecule PAK inhibitor FRAX486 could help treat fragile X syndrome. In the <i>Fmr1</i> knockout mouse model for fragile X syndrome, FRAX486 rescued dendritic spine abnormalities, whereas vehicle did not. In this mouse model, FRAX486 decreased susceptibility to sound-induced seizures, hyperactivity and repetitive behaviors compared with vehicle. Next steps could include evaluating FRAX486 and other PAK inhibitors across multiple fragile X syndrome models.</p> <p>Afraxis Inc. had FRAX486 in preclinical development, and its therapeutics business was acquired by Roche's Genentech Inc. unit in February. Genentech did not disclose its development plans for Afraxis' fragile X compounds.</p> <p>SciBX 6(13); doi:10.1038/scibx.2013.317 Published online April 4, 2013</p>	Patent application filed covering small molecule PAK inhibitors; licensing status unavailable	<p>Dolan, B.M. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online March 18, 2013; doi:10.1073/pnas.1219383110</p> <p>Contact: Susumu Tonegawa, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: tonegawa@mit.edu</p> <p>Contact: Bridget M. Dolan, same affiliation as above e-mail: bdolan@alum.mit.edu</p>
Neuropathy	Transient receptor potential A1 (TRPA1)	<p>Mouse and cell culture studies suggest inhibiting TRPA1 could help prevent chemotherapy-induced sensory neuropathy. In mice that have received Velcade bortezomib or oxaliplatin, TRPA1 antagonists transiently decreased hyperalgesia and allodynia compared with vehicle. In mice, TRPA1 antagonists given before and shortly after treatment with Velcade or oxaliplatin prevented hyperalgesia and allodynia. Next steps could include testing clinical-stage TRPA1 antagonists in additional animal models of chemotherapy-induced neuropathy.</p> <p>Glenmark Pharmaceuticals Ltd.'s TRPA1 antagonist GRC 17536 is in Phase I testing to treat pain.</p> <p>Takeda Pharmaceutical Co. Ltd.'s Millennium Pharmaceuticals Inc. unit and Johnson & Johnson market Velcade, a small molecule dipeptide boronic acid proteasome inhibitor, to treat multiple myeloma (MM) and mantle cell lymphoma (MCL).</p> <p>Oxaliplatin is a generic chemotherapeutic.</p> <p>SciBX 6(13); doi:10.1038/scibx.2013.318 Published online April 4, 2013</p>	Patent and licensing status unavailable	<p>Trevisan, G. <i>et al. Cancer Res.</i>; published online March 11, 2013; doi:10.1158/0008-5472.CAN-12-4370</p> <p>Contact: Pierangelo Geppetti, University of Florence, Florence, Italy e-mail: geppetti@unifi.it</p>

This week in techniques

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

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

Approach	Summary	Licensing status	Publication and contact information
Computational models			
Computational prediction of competitive endogenous RNA (ceRNA) effect on target expression	A computational model of ceRNA function could help identify new drug targets. ceRNAs are transcripts that contain binding sites for the same microRNA, such that competition between the transcripts for miRNA binding can affect their protein expression. Previous studies have shown that ceRNAs can regulate disease targets including the tumor suppressor PTEN (MMAC1; TEP1). The computational model predicted the regulatory effect of interactions between ceRNAs and miRNAs and generated a window of optimal ceRNA-miRNA expression patterns for which meaningful biological effects are likely to occur. Next steps could include using this model to screen for disease-associated ceRNAs.	Patent and licensing status unavailable	Ala, U. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 27, 2013; doi:10.1073/pnas.1222509110 Contact: Pier Paolo Pandolfi, Harvard Medical School, Boston, Mass. e-mail: ppandolf@bidmc.harvard.edu
Drug platforms			
Coagulation factor X (FX)-binding adenovirus 5 (Ad5) for systemic gene delivery	Cell culture and mouse studies suggest FX binding by Ad5 is required for efficient gene therapy with the vector. Prior studies showed that FX increases liver tropism for Ad5 and suggested that engineered viruses lacking FX binding could be useful for systemic gene therapy. In cultured cells incubated in serum, an Ad5 vector engineered to not bind FX had decreased transduction efficiency compared with an FX-binding control vector. In mice lacking antibodies or components of the complement system, the non-FX-binding Ad5 vector had comparable efficiency to an FX-binding Ad5 vector, suggesting that FX helps prevent neutralization of the vector by the complement system. Next steps include exploring how other adenovirus serotypes interact with the complement system.	Unpatented; licensing status not applicable	Xu, Z. <i>et al. Nat. Med.</i> ; published online March 24, 2013; doi:10.1038/nm.3107 Contact: Andrew P. Byrnes, Food and Drug Administration, Bethesda, Md. e-mail: andrew.byrnes@fda.hhs.gov
Crystal structures of serotonin (5-HT) receptor-bound agonists	Crystal structures of agonists bound to 5-HT receptors could aid the development of new compounds selective for 5-HT receptor subtypes. The crystal structures of serotonin (5-HT _{1B}) receptor and serotonin (5-HT _{2B}) receptor bound to ergotamine and dihydroergotamine showed that the small molecules occupy an orthosteric binding pocket on the receptors. Biochemical studies showed that ergotamine preferentially activated noncanonical arrestin- β signaling pathways at 5-HT _{2B} . Next steps include using the crystal structure data to aid the design of compounds specific for different 5-HT receptor subtypes. Ergotamine and dihydroergotamine are both generic migraine drugs.	Findings from both studies unpatented; licensing status not applicable	Wang, C. <i>et al. Science</i> ; published online March 21, 2013; doi:10.1126/science.1232807 Contact: H. Eric Xu, Chinese Academy of Sciences, Shanghai, China e-mail: eric.xu@vai.org Contact: Raymond C. Stevens, The Scripps Research Institute, La Jolla, Calif. e-mail: stevens@scripps.edu
			Wacker, D. <i>et al. Science</i> ; published online March 21, 2013; doi:10.1126/science.1232808 Contact: Bryan L. Roth, The University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, N.C. e-mail: bryan_roth@med.unc.edu Contact: Raymond C. Stevens, The Scripps Research Institute, La Jolla, Calif. e-mail: stevens@scripps.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Structure/function analysis of multidrug and toxic compound extrusion (MATE)-family transporters to guide inhibitor development	Crystallographic analysis of a MATE-family transporter could guide the rational design of inhibitors that circumvent drug resistance mediated by the protein. MATE transporters are conserved from bacteria to humans and export diverse chemical substrates including antibiotics. A MATE transporter from <i>Pyrococcus furiosus</i> was crystallized in multiple conformations including with the fluoroquinolone antibiotic norfloxacin or thioether-macrocylic peptide inhibitors. The structure was used to model the mechanism by which the transporter exports its substrates and imports cations. Next steps include conducting structural studies of MATE transporters from pathogenic bacteria and humans and optimizing the drug-like properties of thioether-macrocylic peptide inhibitors. PeptiDream Inc. discovers macrocylic peptides against targets designated by its partners. SciBX 6(13); doi:10.1038/scibx.2013.322 Published online April 4, 2013	Patent application filed; exclusively licensed to PeptiDream	Tanaka, Y. <i>et al. Nature</i> ; published online March 27, 2013; doi:10.1038/nature12014 Contact: Osamu Nureki, The University of Tokyo, Tokyo, Japan e-mail: nureki@biochem.s.u-tokyo.ac.jp Contact: Hiroaki Suga, same affiliation as above e-mail: hsuga@chem.s.u-tokyo.ac.jp
Trifluoromethyloxadiazole (TFMO)-containing compounds to identify class IIa histone deacetylase (HDAC) inhibitors	TFMO-containing compounds could be used as research compounds to guide development of class IIa HDAC inhibitors. Genetic associations have been identified between class IIa HDACs and Huntington's disease (HD), glucose homeostasis, muscular dystrophies, autoimmunity and ischemic stroke. A screen of about 2 million compounds from GlaxoSmithKline plc's diversity collection identified compounds with HDAC9 inhibitory activity, with 3 of the 4 most potent hits containing the TFMO chemical group. In inhibition assays of HDACs 1–11, the top hit showed preferential inhibition of class IIa HDACs and did not cause cell toxicity. Next steps at Tempero Pharmaceuticals Inc. and GSK include using the research compounds to evaluate the therapeutic potential of class IIa HDACs in autoimmune diseases (<i>see Closer to class IIa HDAC inhibitors, page 1</i>). SciBX 6(13); doi:10.1038/scibx.2013.323 Published online April 4, 2013	Patent status undisclosed; Tempero is interested in collaboration opportunities	Lobera, M. <i>et al. Nat. Chem. Biol.</i> ; published online March 24, 2013; doi:10.1038/nchembio.1223 Contact: Michael A. Nolan, Tempero Pharmaceuticals Inc., Cambridge, Mass. e-mail: mnolan@temperopharma.com
Imaging			
Sequence-specific labeling of multiple cell types in a sample using surface zinc fingers (sZFs) and fluorophore-tagged DNA probes	<i>In vitro</i> studies suggest a method using sZFs and fluorophore-tagged DNA probes could be useful for imaging multiple cell types in complex tissue samples. Cells were labeled with cell surface-binding zinc fingers and then fluorescently tagged with fluorophore probes containing double-stranded DNA specific for a particular sZF. In a mixed population of cells labeled with different sZFs, addition of fluorophore-tagged probes specific for the different sZFs enabled the imaging of individual cell populations as distinct fluorescent signals. Next steps could include using the method to image cells in human tissue samples. SciBX 6(13); doi:10.1038/scibx.2013.324 Published online April 4, 2013	Patent and licensing status unavailable	Mali, P. <i>et al. Nat. Methods</i> ; published online March 17, 2013; doi:10.1038/NMETH.2407 Contact: George M. Church, Harvard Medical School, Boston, Mass. e-mail: gchurch@genetics.med.harvard.edu
Markers			
Highly prevalent telomerase reverse transcriptase (<i>TERT</i>) promoter mutations in tumors including glioblastoma and bladder cancer	Highly prevalent mutations upstream of <i>TERT</i> could help guide the diagnosis and development of new treatments for various cancers. <i>TERT</i> promoter mutations were recently associated in about 70%–80% of melanoma samples, but it was unclear how widespread the mutations were in other cancer types. In a survey of <i>TERT</i> promoter sequences from 1,128 tumors covering 48 tumor types, mutations were identified in 20% of tumors, including 79% of myxoid liposarcomas, 44% of hepatocellular carcinomas, 51% of gliomas and 67% of bladder cancers. In 50 patients with glioblastoma multiforme (GBM), a mutated <i>TERT</i> promoter sequence was associated with lower survival than the wild-type promoter sequence. Next steps could include determining if mutations in the <i>TERT</i> promoter could aid the early diagnosis of various cancers. SciBX 6(13); doi:10.1038/scibx.2013.325 Published online April 4, 2013	Patent and licensing status unavailable	Killela, P.J. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 25, 2013; doi:10.1073/pnas.1303607110 Contact: Hai Yan, Duke University School of Medicine, Durham, N.C. e-mail: yan00002@mc.duke.edu Contact: Bert Vogelstein, The Johns Hopkins University School of Medicine, Baltimore, Md. e-mail: bertvog@gmail.com

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