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Strategies against Xalkori resistance

By Kai-Jye Lou, Staff Writer

Two U.S. groups have uncovered multiple mechanisms in *ALK*-rearranged lung cancers that could drive resistance to **Pfizer Inc.**'s Xalkori crizotinib.^{1,2} The findings suggest Xalkori should be combined with drugs that target other oncogenic pathways such as the *EGFR* and *KIT* pathways, but future studies are needed to determine the frequencies of the identified resistance mechanisms and the best ways to overcome them.

Xalkori is a dual inhibitor of *c-Met* receptor tyrosine kinase (*c-Met*) and anaplastic lymphoma kinase (*ALK*), and their oncogenic variants. Last August, the FDA granted Xalkori accelerated approval to treat advanced or metastatic *ALK*-positive non-small cell lung cancer (NSCLC).

Pfizer originally pursued Xalkori for its *c-Met* inhibition properties but ended up positioning the drug for *ALK*-positive lung cancers after two key developments suggested an *ALK* fusion gene was actually the oncogenic driver in a subset of patients with NSCLC.^{3,4}

The fusion gene, which contains parts of *echinoderm microtubule associated protein like 4 (EML4)* and *ALK*, encodes a cytoplasmic protein with constitutive kinase activity.

In patients with *ALK*-positive NSCLC, Xalkori has produced overall response rates of more than 50%. In contrast, the overall response rate for second-line chemotherapies in lung cancer is only about 10%.

"Crizotinib is one of the most exciting advances in lung cancer in recent years," said Jeffrey Engelman, director of the Center for Thoracic Cancers at **Massachusetts General Hospital Cancer Center** and an assistant professor of medicine at **Harvard Medical School**. "However, despite the high response rate, we find that patients who initially respond to crizotinib usually become resistant to the therapy, and half will become resistant within one year into treatment. We hope that understanding the mechanisms that drive resistance will help give us new insights on how to better treat these patients to overcome resistance or prevent it from developing in the first place."

To elucidate the mechanisms underlying Xalkori resistance, Engelman and colleagues assayed tumor biopsy samples taken from patients with *ALK*-rearranged NSCLC before and after resistance developed.

Analysis of samples from 18 patients with resistance to Xalkori showed that tumors from 5 patients had genomic alterations in *ALK* itself—4 distinct mutations in the tyrosine kinase domain and 1 amplification of the *ALK* fusion gene. However, other patients had *epidermal growth factor receptor (EGFR)* activation, and two samples showed amplification of *stem cell factor receptor tyrosine kinase (c-Kit; KIT; CD117)*.

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“We found that only about 25% of the patients with resistance developed mutations or amplifications in *ALK* itself,” said Engelman. “We did not identify *ALK* mutations in the other 75% of patients who have developed resistance to crizotinib, which suggests that these cancers do not require *ALK* mutations to become resistant to the drug.”

In *ALK*-rearranged human lung cancer cell lines with EGFR- or KIT-mediated Xalkori resistance, Xalkori plus an EGFR or KIT inhibitor decreased proliferation compared with Xalkori alone.

Taken together, the findings suggest the mechanisms that contribute to Xalkori resistance in *ALK*-rearranged NSCLC are diverse and that most such mechanisms do not involve alterations to *ALK* itself.

Results were published in *Science Translational Medicine*.

Layers of complexity

In a separate study, Robert Doebele and colleagues at the **University of Colorado Denver School of Medicine** assayed tumor biopsy samples from 14 *ALK*-rearranged NSCLC patients who showed disease progression while receiving Xalkori.

Like Engelman’s team, the Colorado group identified genetic changes in *ALK* and *EGFR* in patients who developed resistance to Xalkori. In addition, the group detected tumors that lacked an *ALK* gene fusion in three of the patients, which suggests the emergence of tumors driven by non-*ALK* oncogenic pathways. The group also found two cases with activating *K-Ras* mutations.

It is not yet clear whether the *ALK*-negative and *K-Ras*-mutant tumors emerged directly or independently from the *ALK*-rearranged tumors.

“One possibility is that the cancer cells are losing *ALK* and gaining another oncogenic pathway,” said Doebele, who is an assistant professor in the Division of Medical Oncology at the university’s School of Medicine. “Another possibility is that the patient may already have multiple populations of cancer cells with independent oncogenic drivers.”

Data were published in *Clinical Cancer Research*.

“The findings from these two studies represent an important step to understanding *ALK* in lung cancer biology and show that there is an extra layer of complexity in Xalkori resistance on top of mutations in *ALK* itself,” said Timothy Clackson, president of R&D and CSO at cancer company **Ariad Pharmaceuticals Inc.**

He added, “The studies suggest that resistance mechanisms in *ALK*-driven lung cancers, at least for crizotinib, are more complicated than what we typically see in *BCR-ABL*-driven CML [chronic myelogenous leukemia] and *EGFR*-driven cancers,” where resistance typically results from mutations in the receptor tyrosine kinases that are being targeted by the drugs.

Getting testy

The diversity of Xalkori resistance mechanisms suggests repeated tumor profiling in patients with *ALK*-rearranged NSCLC will be important during treatment with the drug.

“The findings from these two studies represent an important step to understanding *ALK* in lung cancer biology and show that there is an extra layer of complexity in Xalkori resistance on top of mutations in *ALK* itself.”

— Timothy Clackson,
Ariad Pharmaceuticals Inc.

Xalkori was approved concurrently with the Vysis ALK Break Apart FISH Probe test from Pfizer's partner **Abbott Laboratories**. The companion molecular diagnostic detects rearrangements involving the *ALK* gene.

But tackling the resistance problem would likely require a separate diagnostic that can screen patient tumors for a series of mechanisms that can drive resistance to Xalkori, including those that do not stem from *ALK* itself.

"It's one thing to look at a single gene for resistance mechanisms, but these studies suggest the need to probe entire pathways with molecular diagnostics, which is going to be more complicated and raises the bar for the design of such diagnostics," said Clackson.

For Xalkori resistance mediated by mutations in the *ALK* kinase domain, Engelman's team reported that distinct *ALK* mutations conferred differential sensitivities to second-generation *ALK* inhibitors.

"Sequencing of the *ALK* gene in tumor DNA will be an important diagnostic method to guide therapeutic decisions and to diagnose resistance mechanisms in the future," said **Tesaro Inc.** president and CSO Mary Lynne Hedley. "These findings suggest the need to isolate and reanalyze patient samples as tumors begin to progress on Xalkori and provide impetus for generating improvements in blood-based and gene-sequencing diagnostics to select the best sequence for treatment with Xalkori and other *ALK* inhibitors that gain regulatory approval."

In March 2011, Tesaro in-licensed **Amgen Inc.**'s preclinical *ALK* inhibitor program.

Strategic insights

In addition to diagnostic insights, the resistance mechanisms also could aid the development of new *ALK* inhibitors.

"Now that we have identified some of these mutations, identifying *ALK* inhibitors that are not subject to such mutations would likely be helpful," said James Christensen, senior director of precision medicine in the division of oncology research at Pfizer. "From a structure-based design perspective, it could be helpful to design inhibitors that block the mutant forms of *ALK*."

"It will also be important to determine whether going in as early as possible with a more potent and more mutation-resistant inhibitor would yield improved patient outcomes in terms of survival and delayed development of resistance," Clackson told *SciBX*.

Because there are many *ALK* mutations that could drive resistance, Doebele thinks companies will need to design inhibitors of multiple mutant forms of the target.

"And for tumors that have become partially or completely independent of *ALK*, one will need to think of combination approaches with other inhibitors such as those targeting the EGFR, KIT or K-Ras pathways," he added.

Pfizer has an ongoing Phase I/II study of Xalkori plus the EGFR inhibitor Tarceva erlotinib in patients with NSCLC. Although the new papers suggest blocking EGFR could overcome Xalkori resistance, the pharma's original rationale for the clinical trial was that Xalkori would help overcome resistance to EGFR inhibitors.

"Our rationale for combining the two drugs was that Xalkori might be able to help overcome or preemptively circumvent the development of c-Met-mediated resistance to EGFR inhibitors," said Christensen. "One thing we're also interested in, initially from a nonclinical perspective,

is alternative dosing schedules for Xalkori itself. For example, can you effectively deliver the drug by pulsing it on an intermittent schedule, and will this help delay the development of resistance?"

The findings also could open up new therapeutic avenues for Ariad's AP26113, a dual inhibitor of *ALK* and EGFR that is in a Phase I/II trial to treat lung cancer. The company plans to start the Phase II portion of the trial this year.

"When we first discovered AP26113, we did not anticipate that its ability to inhibit EGFR could potentially help overcome the mechanisms that drive resistance to *ALK* inhibition," Clackson said. "We originally selected AP26113 for development because it had potential in both EGFR- and *ALK*-driven lung cancers, which are generally viewed as two separate disease subtypes."

Sizable samples

Engelman said his group has expanded its ongoing study to include samples from another 10–12 patients. His group also is working with collaborators to investigate the potential of combining *ALK* inhibitors with other therapeutics to overcome EGFR- and KIT-mediated resistance to Xalkori.

Finally, the group is profiling how the development of resistance to one *ALK* inhibitor correlates with the efficacy of subsequent therapeutic agents, including other *ALK* inhibitors.

"It will be interesting to determine whether the use of more potent *ALK* inhibitors will lead to the development of a different spectrum of point mutations in *ALK* itself," Engelman told *SciBX*.

Doebele said the Colorado group is now trying to overcome the identified resistance mechanisms or prevent them from developing in the first place. He said the group also is searching for and cataloging new resistance mutations and looking at tissue samples for evidence of multiple resistance mechanisms in individual patients.

The findings reported in both papers are unpatented.

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COMPANIES AND INSTITUTIONS MENTIONED

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Amgen Inc. (NASDAQ:AMGN), Thousand Oaks, Calif.
Ariad Pharmaceuticals Inc. (NASDAQ:ARIA), Cambridge, Mass.
Harvard Medical School, Boston, Mass.
Massachusetts General Hospital Cancer Center, Boston, Mass.
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Tesaro Inc., Waltham, Mass.
University of Colorado Denver School of Medicine, Aurora, Colo.

SYKing inhibitors on retinoblastoma

By Chris Cain, Staff Writer

Findings from **St. Jude Children's Research Hospital** and **Washington University in St. Louis** researchers show that spleen tyrosine kinase is epigenetically upregulated in retinoblastoma, suggesting that blocking its activity could help treat the disease.¹ The results provide a potential new indication for spleen tyrosine kinase inhibitors, and the researchers are already working on an ocular formulation of one.

Retinoblastoma is a rare childhood cancer of the eye most commonly caused by inactivating mutations in the *retinoblastoma 1* (*RB1*) tumor suppressor gene. Although more than 95% of children in the U.S. are cured of the disease, treatment requires chemotherapy or localized radiation therapy, and patients who do not adequately respond are at risk for vision loss. In developing nations the prognosis is worse, as the cancer can metastasize if not caught early.

In 2010, St. Jude and Washington University formed the Pediatric Cancer Genome Project, a three-year collaboration to sequence the genomes of about 600 pediatric cancer patients, including those with retinoblastoma.

Because *RB1* is a known regulator of essential cellular processes including DNA methylation, histone modification and cell cycle progression, researchers involved in the project suspected they would find genetic changes that might point the way to new targets.

Surprisingly, the team did not find a striking amount of genetic change in the four sequenced primary retinoblastoma samples or in an orthotopic retinoblastoma xenograft that had grown in mice for more than nine months. The rates of mutation and chromosomal structural alterations were lower than those seen in many other tumor types.

The lack of genetic changes prompted the researchers to look more closely at genomewide epigenetic changes, including histone and DNA methylation patterns, to help explain how loss of *RB1* drives retinoblastoma growth.

The team combined genomewide expression data with an analysis of histone modification and DNA methylation to pinpoint candidate genes that had significant changes in patient samples compared with normal fetal retinas. *Spleen tyrosine kinase* (*SYK*) came out of the integrated computational analysis as the top kinase hit.

"*SYK* was upregulated in the gene expression analysis, but it was not our top candidate until we did the complete epigenetic analysis," said Michael Dyer, a senior author of the study.

He said *SYK* is expressed in blood cells that often contaminate tumor samples and is not normally expressed in the eye. Thus, the team would have considered it a false positive without the epigenetic data to back it up.

Dyer is coleader of the Developmental Therapeutics for Solid Malignancies Program and director of the Division of Developmental Biology at St. Jude.

The group next assessed the functional role of *SYK* in retinoblastoma. *SYK* is normally expressed in immune cells and is a regulator of inflammation, but it has also been suggested as a target in hematological malignancies because it can promote cell survival.

In cultured retinoblastoma cells, small hairpin RNA knockdown or pharmacological inhibition of *SYK* induced cell death compared with no knockdown or treatment. In an orthotopic xenograft model of retinoblastoma, topotecan plus ocular injection of a *SYK* inhibitor prolonged survival compared with topotecan alone.

Results were published in *Nature*.

"The beauty of this story is that because there were hardly any genetic lesions, we could dig into the epigenetic analysis and look for specific changes. It would have been very hard to sort out what the contributing genetic and epigenetic factors were if you had thousands of point mutations and hundreds of chromosomal translocations on top of these epigenetic changes," said Dyer.

Joan O'Brien, chair of the Department of Ophthalmology at the Scheie Eye Institute of the **University of Pennsylvania**, told *SciBX*, "The preclinical data provided here are indeed suggestive that *SYK* inhibition could play

a clinical role in treatment of retinoblastoma. More specific targeted therapies are required for this disease because neonatal patients with germline retinoblastoma experience an increased incidence of second tumors as well as side effects from today's treatment regimens."

She added that the lack of genetic variation in these samples is a surprising finding and further studies are needed to reconcile this study with previous work. Previous studies in cell culture had also suggested loss of *RB1* could lead to widespread chromosomal structural alterations.²

Dyer said experiments are ongoing to explain the apparent discrepancy between those studies and what his team found in primary tumor samples.

David Cobrinik, a senior research scientist at **Memorial Sloan-Kettering Cancer Center**, said that sequencing additional samples is necessary to confirm that there is consistently a low level of genomic structural alteration. He also cautioned that the high levels of *SYK* could reflect *SYK* expression in a rare retinal cell type, as well as the adoption of an altered epigenetic state.

"We are very up front in the paper that we have no idea what the mechanism of *SYK* upregulation is," Dyer told *SciBX*. He said *RB1* could be acting to directly regulate *SYK* transcription or acting indirectly by globally changing chromatin structure.

Dyer's lab is now studying the pathways upstream and downstream of *SYK* in retinoblastoma and is investigating *SYK* expression in metastatic retinoblastoma samples.

Targeting SYK

Dyer also is developing an ocular formulation of **Rigel Pharmaceuticals Inc.**'s R406 small molecule *SYK* inhibitor, which is available as a research reagent. His group has developed a pharmacokinetics assay to detect R406 in the plasma, retina and vitreous of the eye and plans to also examine ocular toxicity.

"The beauty of this story is that because there were hardly any genetic lesions, we could dig into the epigenetic analysis and look for specific changes."

—Michael Dyer,
St. Jude Children's Hospital

Dyer has no specific timeline for starting a Phase I trial of the formulation.

Rigel's fostamatinib (R788), an oral prodrug formulation of R406 that is partnered with **AstraZeneca plc**, is in Phase III testing to treat rheumatoid arthritis (RA) and in Phase II trials to treat diffuse large B cell lymphoma (DLBCL).

"AstraZeneca is committed to developing a greater understanding of SYK in a range of pathologies," but the pharma has no plans for clinical studies in other disease areas at this time, according to spokesperson Sameena Conning.

Other SYK inhibitors in clinical development include the oral small molecules PRT062607 from **Portola Pharmaceuticals Inc.** and partner **Biogen Idec Inc.**, and **Gilead Sciences Inc.**'s GS-9973. PRT062607 has completed Phase I testing, and the partners plan to begin a Phase IIa trial in RA in 2H12. GS-9973 is in Phase I testing for RA. The three companies declined to comment on the *Nature* results.

Also in development is **ZaBeCor Pharmaceutical Co.**'s Excellair small interfering RNA targeting SYK, which has completed a Phase II trial to treat asthma.

ZaBeCor founder, chairman and CEO Alan Schreiber told *SciBX* that studies have shown inhibiting SYK is effective in preventing cancer cell proliferation and noted that siRNA could be an effective way to deliver therapeutics to the eye. He said the company has performed preclinical studies demonstrating the use of siRNA to inhibit SYK activity in mouse models of ocular inflammation.

ZaBeCor owns U.S. and international patents covering the use of its SYK-targeting siRNA technology to target any human organ. The company is looking for partners to develop its technology in retinoblastoma and other indications.

In addition to his work on SYK, Dyer is developing two other treatments for retinoblastoma targeting distinct pathways altered by loss of *RB1*.

Changes in genomewide histone acetylation linked to loss of *RB1* have suggested that patients with retinoblastoma may benefit from treatment with histone deacetylase (HDAC) inhibitors.³ Separately, overexpression of the survival factors mdm2 p53 binding protein homolog (MDM2; HDM2) and MDM4 (MDMX) in patients with retinoblastoma suggested a use for MDM inhibitors.⁴

"We have three possible approaches here, so we're going to get all the data and sit down and decide how to proceed based on efficacy, toxicity and route to the clinic," Dyer said. He added that once the data package is assembled, he wants to look for corporate partnerships.

The findings reported in *Nature* are unpatented.

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COMPANIES AND INSTITUTIONS MENTIONED

AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
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Steroids not so depressing

By **Tim Fulmer**, Senior Writer

Mapreg S.A.S. researchers have shown for the first time *in vivo* that targeting microtubule-associated protein 2 with steroid derivatives can have an antidepressant effect in mouse models of depression.¹ Indeed, in some of those models, the company's MAP4343 had an onset of action that was faster than that for Prozac fluoxetine.

Mapreg founder, president and CEO Etienne Baulieu identified 3-methoxy-pregnenolone (MAP4343) in a screen for steroid derivatives that altered the function of microtubules in neurons. In 2006, Mapreg published data showing that the compound bound microtubule-associated protein 2 (MAP2) *in vitro* and improved recovery of locomotor function in rat models of spinal cord injury (SCI).^{2,3}

Two years later, MAP4343 received EU Orphan Drug designation for SCI and started Phase I testing. Also in 2008, Baulieu came across a paper by researchers at **The University of Nottingham Medical School** that showed altered MAP2 levels in the hippocampus were accompanied by abnormal synaptic connections and depressive-like behavior.^{4,5}

He hired corresponding author Massimiliano Bianchi as director of R&D psychopharmacology, and Mapreg has now confirmed the hypothesis that MAP4343 acts as an antidepressant by blocking MAP2 activity in the hippocampus and prevents depression-associated tissue pathology.

Indeed, in mice, a single subcutaneous dose of MAP4343 was able to cross the blood brain barrier, enter the hippocampus and induce changes in isoforms of tubulin- α , the endogenous substrate of MAP2.

Next, the company studied MAP4343 in the rat forced swim test, an assay used to screen for *in vivo* antidepressant activity. Animals receiving 4 mg and 10 mg doses of MAP4343 showed significantly decreased passive coping behavior compared with rats given vehicle ($p < 0.05$ and $p < 0.001$, respectively). High levels of passive coping behavior are a sign of depression.

The low dose had similar activity to a 10 mg dose of Prozac, which is marketed by **Eli Lilly and Co.** to treat major depressive disorder, obsessive-compulsive disorder (OCD), bulimia nervosa and panic disorder.

Bianchi declined to disclose how the dosing used in the depression models compared with the dosing used in the SCI trials.

Eli Lilly did not respond to requests for comment.

In a rat model of acute and chronic depression, MAP4343 given for 1–4 days significantly decreased short-term passive coping behavior compared with vehicle ($p < 0.01$), whereas Prozac showed no effect. In the same rats, both MAP4343 and Prozac reduced coping behavior and anxiety at 7–10 days of treatment compared with vehicle ($p < 0.001$ and $p < 0.05$, respectively).

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

Faster than Prozac

MAP4343's speed in the depression setting is intriguing, as first-line drugs such as Prozac and other selective serotonin reuptake inhibitors (SSRIs) can have a lag of 2–3 weeks between the start of treatment and clinical improvement.⁶

To better determine the time window of MAP4343's rapid antidepressant effects, Ronald Duman wanted to see MAP4343 tested in a rat model of depression that measures how acute and chronic stress induce anhedonia (the inability to experience pleasure) over time.⁷

Duman is professor of psychiatry, neurobiology and pharmacology and director of the Division of Molecular Psychiatry Abraham Ribicoff Research Facilities at **Yale University**.

"To get a better idea of how rapidly MAP4343 works in depression, it would be useful to compare the short-term antidepressant effects of MAP4343 and ketamine in depression models," said Martin Beaulieu, assistant professor of psychiatry and neuroscience at **Laval University**.

In pilot trials, low doses of ketamine have shown antidepressant effects within two hours, with relief lasting for several days.⁸

However, higher doses can trigger severe hallucinogenic and psychotic symptoms. Moreover, ketamine is a Schedule III controlled substance, which could complicate its use in depression.

Beaulieu cautioned that it will be "important to get a better idea of the specificity of this steroid derivative for MAP2 in the hippocampus, since various MAP isoforms occur throughout the CNS, where they help maintain microtubule stability. If MAP4343 were to hit some of those other isoforms too, there could be toxicity associated with impairing processes like axonal transport."

Bianchi said MAP4343 does not hit targets other than MAP2. "The compound was screened for *in vitro* affinity to 80 different neurotransmitter and steroid receptors and essentially negative results were obtained," he noted.

Moving forward, Mapreg "will continue to study MAP4343 for its possible use in other CNS alterations associated with aging and psychiatric disorders," said Baulieu, who was corresponding author on the paper. "Other derivatives of pregnenolone are also being studied."

Mapreg hopes to start a Phase II trial in SCI this year.

Baulieu and colleagues have applied for a U.S. patent covering the use of 3-methoxy-pregnenolone to treat depressive disorders and long-term neurological diseases. The IP is available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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By Lev Osherovich, Senior Writer

Researchers at the NIH have proposed a new mechanism to explain the beneficial metabolic effects of resveratrol, a polyphenol compound from red wine thought by many to act primarily on sirtuin 1. The NIH team suggests resveratrol works by inhibiting phosphodiesterase-4, an enzyme that sits far upstream of sirtuin 1 and affects a signaling pathway that leads to increased energy utilization.¹

The findings could provide repurposing opportunities for the plethora of phosphodiesterase-4 (PDE-4) inhibitors on the market or in the clinic, primarily for pulmonary indications.

Prior work by researchers at **Harvard University** and the **Massachusetts Institute of Technology** suggested resveratrol promotes the consumption of energy and other antidiabetic effects by activating sirtuin 1 (SIRT1), a protein deacetylase implicated in a variety of metabolic and neurodegenerative processes.² Indeed, that research led to the formation of **Sirtris Pharmaceuticals Inc.**, which was acquired in 2008 by **GlaxoSmithKline plc** for \$651 million.

In 2010, GSK discontinued development of Sirtris' lead compound, SRT501, after data from an open-label Phase IIa trial in 24 patients showed the orally bioavailable formulation of resveratrol had minimal

Figure 1. Explaining resveratrol's metabolic effects. Park *et al.* propose that the principal target of resveratrol is phosphodiesterase 4 (PDE-4), not sirtuin 1 (SIRT1) as previously thought.

The team found that resveratrol can inhibit PDE-4, an enzyme that degrades cyclic AMP (cAMP) in response to signaling by glucagon.

Glucagon is a peptide hormone that stimulates metabolic activity. Normally, extracellular glucagon works through glucagon receptor (GCGR) [a] to stimulate adenylate cyclase 1 (ADCY1; AC1) to convert ATP into cAMP, which in turn stimulates rap guanine nucleotide exchange factor 3 (RAPGEF3; EPAC) [b]. Higher EPAC activity indirectly leads to activation of AMP-activated protein kinase (AMPK) [c], a key regulator of cellular energy utilization.

Park *et al.* report that resveratrol inhibited PDE-4 *in vitro* and stimulated EPAC activity and AMPK phosphorylation in cell culture [d]. In a mouse model of diet-induced obesity, the generic PDE-4 inhibitor rolipram increased AMPK activity [e] and SIRT1 activation [f]. Based on these observations, Park *et al.* suggest the metabolic effects of resveratrol are likely due to PDE-4 inhibition.

At least 16 PDE-4 inhibitors are marketed or in development for a range of inflammatory and pulmonary indications, including Daliresp roflumilast from **Takeda Pharmaceutical Co. Ltd.**, **Mitsubishi Tanabe Pharma Corp.** and **Forest Laboratories Inc.**, which is marketed to treat chronic obstructive pulmonary disease (COPD), and Ketas ibudilast from **Kyorin Pharmaceutical Co. Ltd.**, which is marketed in Japan to treat asthma and stroke.

Betagenon AB and **Connexios Life Sciences Pvt. Ltd.** have AMPK activators in preclinical development for type 2 diabetes and obesity. AMPK also is the principal target of metformin, a generic drug to treat type 2 diabetes.

In 2010, **GlaxoSmithKline plc** discontinued development of SRT501, an orally bioavailable formulation of resveratrol, after data from an open-label Phase IIa trial in 24 patients showed the compound had minimal efficacy and the potential to indirectly exacerbate renal complications in patients with multiple myeloma (MM).

The pharma's SIRT1 activator SRT2104 has completed Phase II testing for type 2 diabetes and is in Phase I testing for various cardiovascular and inflammatory indications. GSK has at least two other SIRT1 activators in Phase I testing.

efficacy and increased the risk of renal complications in patients with multiple myeloma (MM).

The pharma's next-generation SIRT1 activator, SRT2104, has completed Phase II testing for type 2 diabetes and is in Phase I testing for cardiovascular and inflammatory indications. GSK has at least two other SIRT1 activators in Phase I testing in inflammatory indications.

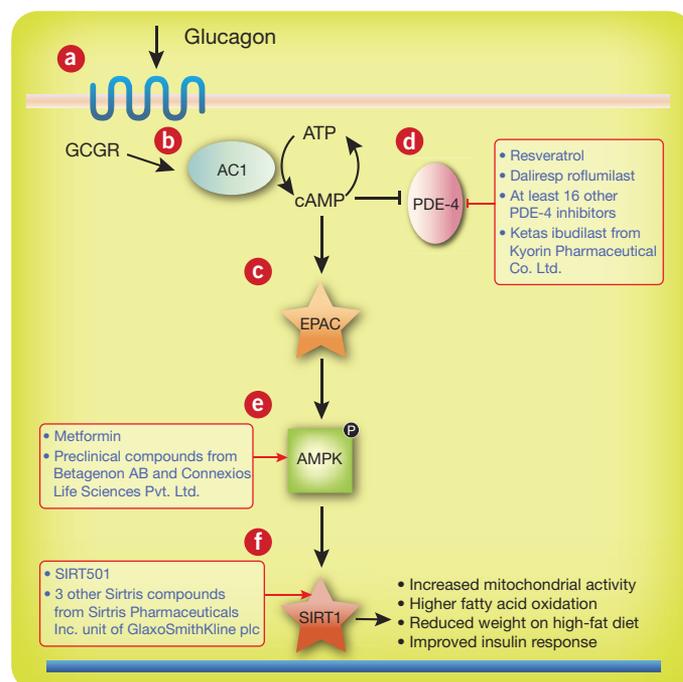
While the molecules were moving through the clinic, independent teams at several academic institutions and at **Elixir Pharmaceuticals Inc.**, **Amgen Inc.** and **Pfizer Inc.** reported that, in their hands, resveratrol and three other Sirtris compounds, not including SRT2104, did not directly activate SIRT1 *in vitro*.³⁻⁶

"The question is how can these compounds activate SIRT1 if they don't directly activate it," said Jay Chung, chief of the Laboratory of Obesity and Aging Research at the NIH's **National Heart, Lung, and Blood Institute**.

New findings from Chung's group point to PDE-4 as a potential target of resveratrol. "Our findings elucidate one possible mechanism for resveratrol's action," said Chung.

To PDE or not to PDE?

Chung's team examined the metabolic effects of resveratrol in mice and in cultured mammalian cells. Compared with vehicle, resveratrol raised levels of cyclic AMP (cAMP), a signaling molecule that activates



metabolic pathways in response to signaling by glucagon. Glucagon is a peptide hormone that promotes energy utilization.

Building on previous studies by other teams suggesting that resveratrol can inhibit phosphodiesterases,⁷ a class of cAMP-degrading enzymes, Chung hypothesized that resveratrol inhibition of phosphodiesterases could account for the compound's effect on cAMP.

To test the idea, the group screened a panel of phosphodiesterases and found resveratrol potently blocked PDE-4. There are two marketed PDE-4 inhibitors: Daliresp roflumilast, a selective compound from **Takeda Pharmaceutical Co. Ltd.**, **Mitsubishi Tanabe Pharma Corp.** and **Forest Laboratories Inc.** marketed to treat chronic obstructive pulmonary disease (COPD), and Ketas ibudilast, a nonselective compound marketed in Japan by **Kyorin Pharmaceutical Co. Ltd.** to treat asthma and stroke. At least 16 other PDE-4 inhibitors are in development.

Chung's team worked its way down the pathway and showed that resveratrol could turn on AMP-activated protein kinase (AMPK), a central metabolic regulator that in turn activates SIRT1 (see **Figure 1**, "Explaining resveratrol's metabolic effects").

In cell culture, a generic PDE-4 inhibitor increased cAMP levels, AMPK signaling and SIRT1 activity compared with vehicle. In a mouse model of diet-induced obesity, the PDE-4 inhibitor modestly decreased body fat and glucose tolerance compared with vehicle.

Results were reported in *Cell* and are unpatented.

"We believe that these compounds indirectly activate SIRT1 by inhibiting phosphodiesterases," said Chung. "We have identified the pathway by which cAMP can confer metabolic benefits, including SIRT1 activation."

George Vlasuk, CEO of GSK's Sirtris unit, told *SciBX* he thinks Chung's conclusions are based on circumstantial evidence.

Chung's team "investigated the effects of a selective PDE-4 inhibitor in a mouse model of diet-induced obesity and proceeded to circumstantially link the beneficial effects of this compound in this setting to the inhibition of phosphodiesterase activity by resveratrol," said Vlasuk.

He thinks that because Chung's team did not do a direct comparison of the two compounds in the same models, "one cannot link the observed metabolic benefits of both PDE-4 inhibition and resveratrol."

Chung said that because resveratrol interacts with more phosphodiesterases than just PDE-4, he would not expect PDE-4 inhibition to exactly match resveratrol's effects. He added that the data in the paper show that, at least qualitatively, PDE-4 inhibition reproduces the overall effects of treatment with resveratrol.

Vlasuk also said the plasma concentration needed to achieve beneficial effects with resveratrol is much lower than the concentration of resveratrol needed to inhibit PDE-4 *in vitro*. Thus, he suspects that if resveratrol does indeed inhibit phosphodiesterases, this activity does not account for its metabolic effects.

Matt Kaerberlein, associate professor of pathology at the **University of Washington**, is on the fence about Chung's hypothesis. Kaerberlein has previously published evidence against resveratrol's direct activation of SIRT1.⁴ "People have speculated that some aspects of resveratrol's activity are mediated through AMPK, whether directly or not," he said.

Kaerberlein added that Chung's findings "fit with the idea that there may be a target for resveratrol upstream of AMPK, but I'm skeptical that PDE-4 accounts for all the effects of resveratrol."

Indeed, because resveratrol is a fairly promiscuous molecule that binds to multiple targets, "we cannot prove that there is no other mechanism," said Chung.

Additive or synergistic

One way to test Chung's hypothesis would be to combine resveratrol and a PDE-4 inhibitor to see whether the two compounds have an additive effect, as would be expected for a common target, or a synergistic effect, as would be expected for separate targets.

Chung told *SciBX* that such an experiment could be informative.

Ultimately, Chung said, the promiscuous nature of resveratrol and the complex regulation of metabolism make it hard to nail down how the molecule really works. "It is not clear whether there is a definitive experiment that can resolve this question to everyone's satisfaction," he said.

Kaerberlein agreed, noting that a different approach to the question of resveratrol's target could come from experiences in the clinic. He suggested that Chung's team "look in patients who are already being treated with PDE-4 inhibitors to see if there's protection against diabetes."

Chung may soon get such data. He plans to conduct a clinical trial of an undisclosed PDE-4 inhibitor in obesity and metabolic syndrome in partnership with an undisclosed pharma company.

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COMPANIES AND INSTITUTIONS MENTIONED

Amgen Inc. (NASDAQ:AMGN), Thousand Oaks, Calif.
Elixir Pharmaceuticals Inc., Cambridge, Mass.
Forest Laboratories Inc. (NYSE:FRX), New York, N.Y.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Harvard University, Cambridge, Mass.
Kyorin Pharmaceutical Co. Ltd. (Tokyo:4569), Tokyo, Japan
Massachusetts Institute of Technology, Cambridge, Mass.
Mitsubishi Tanabe Pharma Corp. (Tokyo:4508; Osaka:4508), Osaka, Japan
National Heart, Lung, and Blood Institute, Bethesda, Md.
National Institutes of Health, Bethesda, Md.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Sirtris Pharmaceuticals Inc. (NASDAQ:SIRT), Cambridge, Mass.
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan
University of Washington, Seattle, Wash.

"We believe that these compounds indirectly activate SIRT1 by inhibiting phosphodiesterases. We have identified the pathway by which cAMP can confer metabolic benefits, including SIRT1 activation."

—Jay Chung,
*National Heart, Lung,
and Blood Institute*

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				
Breast cancer	Cathepsin B	<p>Mouse studies suggest inhibiting cathepsin B could help treat or prevent breast cancer metastasis. In a mouse model of breast cancer, cathepsin B small hairpin RNA decreased lung and bone metastasis compared with control shRNA but did not alter growth of the primary tumor. A cathepsin B-specific inhibitor lowered metastasis compared with vehicle control or a broad-spectrum cysteine protease inhibitor. Next steps include designing a more selective cathepsin B inhibitor.</p> <p>SciBX 5(7); doi:10.1038/scibx.2012.171 Published online Feb. 16, 2012</p>	Unpatented; licensing status unavailable	<p>Withana, N.P. <i>et al. Cancer Res.</i>; published online Jan. 19, 2012; doi:10.1158/0008-5472.CAN-11-2759 Contact: Belinda S. Parker, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia e-mail: belinda.parker@petermac.org</p>
Cancer	CD44; pyruvate kinase M2 isozyme (PKM2)	<p>Cell culture studies suggest inhibiting CD44 could help sensitize glycolytic cancer cells to chemotherapy. In <i>p53</i>-deficient cancer cells undergoing aerobic glycolysis, small interfering RNA against CD44, which directly interacts with the glycolysis regulator PKM2, increased PKM2 activity and decreased aerobic glycolysis compared with control siRNA. In the same cells, combining the generic chemotherapeutic cisplatin with siRNA targeting CD44 increased cell death compared with cisplatin plus control siRNA. Next steps include identifying inhibitors that disrupt the interaction of CD44 and PKM2.</p> <p>Agios Pharmaceuticals Inc. and Dynamix Pharmaceuticals Ltd. have independent preclinical programs targeting PKM2 for cancer.</p> <p>SciBX 5(7); doi:10.1038/scibx.2012.172 Published online Feb. 16, 2012</p>	Unpatented; unavailable for licensing	<p>Tamada, M. <i>et al. Cancer Res.</i>; published online Jan. 31, 2012; doi:10.1158/0008-5472.CAN-11-3024 Contact: Hideyuki Saya, Keio University School of Medicine, Tokyo, Japan e-mail: hsaya@a5.keio.jp</p>
Cancer	Not applicable	<p>Mouse studies suggest nutrient starvation could increase the efficacy of cancer chemotherapy. In xenograft and allograft mouse models of cancer, mice that fasted for 48–60 hours had less tumor growth than mice that did not fast. In mice, doxorubicin or cyclophosphamide chemotherapy plus fasting further decreased tumor growth compared with chemotherapy alone. In mouse models of metastatic cancer, fasting plus chemotherapy prolonged survival compared with chemotherapy alone. Next steps include running clinical trials to test the effects of fasting or food products from L-Nutra Inc. on chemotherapy efficacy and seeing if fasting can decrease the number of side effects.</p> <p>L-Nutra, a medicinal food company developing diets for chemotherapy patients, holds rights to the findings.</p> <p>SciBX 5(7); doi:10.1038/scibx.2012.173 Published online Feb. 16, 2012</p>	Patent applications filed; exclusively licensed to L-Nutra	<p>Lee, C. <i>et al. Sci. Transl. Med.</i>; published online Feb. 8, 2012; doi:10.1126/scitranslmed.3003293 Contact: Valter D. Longo, University of Southern California, Los Angeles, Calif. e-mail: vlongo@usc.edu</p>

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Colorectal cancer	Epidermal growth factor receptor (EGFR)	<p>Cell culture and patient studies suggest patients with colorectal cancer (CRC) who become resistant to Erbitux cetuximab as a result of an S492R EGFR mutation may respond to Vectibix panitumumab. In S492R EGFR-mutant CRC cell lines, Vectibix blocked receptor activation, whereas Erbitux did not. In 10 patients with CRC who had disease progression while receiving Erbitux, 2 had an S492R EGFR mutation that was not present prior to treatment and 1 subsequently responded to Vectibix. Next steps could include conducting a prospective clinical trial to assess S492R EGFR as a marker for Vectibix treatment in CRC and determining if the mutation contributes to Erbitux resistance in additional cancers.</p> <p>Erbitux, a mAb targeting EGFR from Eli Lilly and Co., Bristol-Myers Squibb Co. and Merck KGaA, is marketed to treat colorectal cancer and head and neck cancer.</p> <p>Vectibix, a mAb against EGFR1 (HER1; ERBB1) from Amgen Inc. and Takeda Pharmaceutical Co. Ltd., is marketed to treat colorectal cancer.</p> <p>SciBX 5(7); doi:10.1038/scibx.2012.174 Published online Feb. 16, 2012</p>	Patent application filed; available for licensing	<p>Montagut, C. <i>et al. Nat. Med.</i>; published online Jan. 22, 2012; doi:10.1038/nm.2609</p> <p>Contact: Joan Albanell, Hospital del Mar, Barcelona, Spain e-mail: jalbanell@hospitaldelmar.cat</p> <p>Contact: Clara Montagut, same affiliation as above e-mail: cmontagut@hospitaldelmar.cat</p>
Gastrointestinal cancer	MicroRNA-196a (miR-196a); HOX transcript antisense RNA (HOTAIR)	<p>Patient sample and cell culture studies suggest miR-196a or HOTAIR could be prognostic markers or therapeutic targets for gastrointestinal cancer. Patients with high levels of miR-196a or HOTAIR in gastrointestinal stromal tumors (GISTs) had significantly shorter survival times than patients with low levels. In GIST cells, small interfering RNA against HOTAIR decreased cell viability and invasion compared with control siRNA. Next steps include measuring levels of miR-196a in the blood of patients with GIST and confirming the relationship between patient prognosis and expression of HOTAIR and miR-196a in a prospective clinical trial.</p> <p>SciBX 5(7); doi:10.1038/scibx.2012.175 Published online Feb. 16, 2012</p>	Patent application filed; available for licensing	<p>Niinuma, T. <i>et al. Cancer Res.</i>; published online Jan. 18, 2012; doi:10.1158/0008-5472.CAN-11-1803</p> <p>Contact: Hiromu Suzuki, Sapporo Medical University School of Medicine, Hokkaido, Japan e-mail: hsuzuki@sapmed.ac.jp</p>
Prostate cancer	Protein tyrosine phosphatase 1B (PTP-1B; PTPN1)	<p>Studies in mice and in patient samples suggest inhibiting PTP-1B could help treat prostate cancer. In samples from 218 prostate cancer patients, PTP-1B amplification was found in 21.6% of metastases compared with 6.6% of primary tumors. In mice, prostate cancer xenografts expressing PTP-1B shRNA had less tumor growth than xenografts expressing control shRNA. Next steps include identifying other mutations that could influence the progression of prostate cancers with high PTP-1B expression and determining whether blocking PTP-1B could improve clinical outcomes.</p> <p>TransTech Pharma Inc.'s PTP-1B inhibitor, TTP814, is in Phase II testing to treat diabetes.</p> <p>ISIS-PTP1BRx, a PTP-1B antisense oligonucleotide from Isis Pharmaceuticals Inc., is in Phase I testing to treat diabetes.</p> <p>MSI-1436, a small molecule PTP-1B inhibitor from Ohr Pharmaceutical Inc., is in Phase I testing to treat diabetes.</p> <p>SciBX 5(7); doi:10.1038/scibx.2012.176 Published online Feb. 16, 2012</p>	Unpatented; vectors, cell lines and animal models developed in the project are available for licensing from McGill University	<p>Lessard, L. <i>et al. Cancer Res.</i>; published online Jan. 26, 2012; doi:10.1158/0008-5472.CAN-11-2602</p> <p>Contact: Michel L. Tremblay, McGill University, Montreal, Quebec, Canada e-mail: michel.tremblay@mcgill.ca</p>

This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cardiovascular disease				
Aneurysm	MicroRNA-29b (miR-29b)	Studies in mice suggest inhibiting miR-29b could help treat abdominal aortic aneurysm. In a mouse model of the condition, overexpression of miR-29b led to increased progression and delivery of a miR-29b antagomir decreased disease progression compared with what was seen using placebo. Next steps include exploring methods for delivering an miR-29b antagomir to the site of injury as well as assessing different formulations to increase duration of the therapeutic effect. SciBX 5(7); doi:10.1038/scibx.2012.177 Published online Feb. 16, 2012	Findings covered by pending patents; available for licensing from Stanford University	Maegdefessel, L. <i>et al. J. Clin. Invest.</i> ; published online Jan. 24, 2012; doi:10.1172/JCI61598 Contact: Philip S. Tsao, Stanford University School of Medicine, Palo Alto, Calif. e-mail: ptsao@stanford.edu
Aneurysm	Properdin	Studies in mice suggest inhibiting properdin could help treat abdominal aortic aneurysm. Properdin is a protein involved in the proinflammatory complement alternative pathway. In mouse models of abdominal aortic aneurysm, properdin deficiency prevented the condition compared with wild-type properdin expression. An anti-properdin antibody also prevented abdominal aortic aneurysm compared with control antibody. Next steps include further studies of the role properdin plays in the development of abdominal aortic aneurysm. SciBX 5(7); doi:10.1038/scibx.2012.178 Published online Feb. 16, 2012	Unpatented; licensing status not applicable	Zhou, H.-f. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Jan. 30, 2012; doi:10.1073/pnas.1119000109 Contact: Dennis E. Hourcade, Washington University in St. Louis, St. Louis, Mo. e-mail: dhourcad@dom.wustl.edu
Endocrine/metabolic disease				
Obesity; diabetes; metabolic syndrome	Phosphodiesterase-4 (PDE-4)	Studies in cell culture and in mice suggest inhibiting PDE-4 could help treat obesity and type 2 diabetes. In mice fed a high-fat diet, a PDE-4 inhibitor increased metabolic activity and lowered blood glucose levels and weight gain compared with vehicle. In cell culture, the inhibitor increased activity of AMP-activated protein kinase (AMPK), a regulator of metabolic pathways involved in energy utilization, compared with vehicle. Next steps include clinical testing of PDE-4 inhibitors in metabolic indications. At least 15 companies have PDE-4 inhibitors in development or marketed for various indications such as inflammation and autoimmune diseases (<i>see Still un-sirtuin, page 8</i>). SciBX 5(7); doi:10.1038/scibx.2012.179 Published online Feb. 16, 2012	Unpatented; licensing status not applicable	Park, S.-J. <i>et al. Cell</i> ; published online Feb. 3, 2012; doi:10.1016/j.cell.2012.01.017 Contact: Jay H. Chung, National Institutes of Health, Bethesda, Md. e-mail: chungj@nhlbi.nih.gov
Infectious disease				
Bacterial infection	β -Lactamase (LACTB)	Cell culture studies identified a noncovalent inhibitor of class A LACTBs that could help treat drug-resistant bacterial infections. In two cephalosporin-resistant clinical <i>Escherichia coli</i> isolates, the lead Lactb inhibitor increased sensitivity to the cephalosporin antibiotic cefotaxime compared with no inhibitor. Next steps include testing the lead inhibitor against additional LACTBs from clinically isolated bacteria strains and designing and synthesizing analogs with broad-spectrum activity. Cefotaxime is a generic broad-spectrum cephalosporin antibiotic. At least eight companies have LACTB inhibitors in development stages from preclinical to marketed to treat bacterial infections. SciBX 5(7); doi:10.1038/scibx.2012.180 Published online Feb. 16, 2012	Provisional patent application filed covering compounds and their analogs; licensing status unavailable	Nichols, D.A. <i>et al. J. Med. Chem.</i> ; published online Feb. 1, 2012; doi:10.1021/jm2014138 Contact: Yu Chen, University of South Florida, Tampa, Fla. e-mail: ychen1@health.usf.edu Contact: Adam R. Renslo, University of California, San Francisco, Calif. e-mail: adam.renslo@ucsf.edu

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
HIV/AIDS	HIV gp120; CC chemokine receptor 5 (CCR5; CD195); CXC chemokine receptor 4 (CXCR4; NPY3R)	<i>In vitro</i> and cell assay studies identified a dual CD4-heparin sulfate peptide (mCD4-P3YSO ₃) that could help treat HIV. <i>In vitro</i> , mCD4-P3YSO ₃ blocked HIV gp120 from binding to host CCR5 and CXCR4. In cellular assays, mCD4-P3YSO ₃ inhibited viral replication of two HIV-1 reference strains at low nM doses. Next steps include testing mCD4-P3YSO ₃ and other mCD4-linked heparin sulfate mimetic peptides in animal models. SciBX 5(7); doi:10.1038/scibx.2012.181 Published online Feb. 16, 2012	Patented; available for licensing	Connell, B.J. <i>et al. Chem. Biol.</i> ; published online Jan. 27, 2012; doi:10.1016/j.chembiol.2011.12.009 Contact: Hugues Lortat-Jacob, University Joseph Fourier, Grenoble, France e-mail: hugues.lortat-jacob@ibs.fr
Trypanosome	Tryparedoxin	<i>In vitro</i> studies suggest inhibiting tryparedoxin could help treat trypanosome infections. In cultured <i>Trypanosoma brucei</i> , 12 compounds that were identified from a high throughput screen inhibited growth with low- to submicromolar EC ₅₀ values. Six of the compounds were shown to covalently modify tryparedoxin. Next steps could include optimizing and evaluating the lead compounds in animal models of trypanosome infection. SciBX 5(7); doi:10.1038/scibx.2012.182 Published online Feb. 16, 2012	Patent and licensing status unavailable	Fueller, F. <i>et al. J. Biol. Chem.</i> ; published online Jan. 23, 2012; doi:10.1074/jbc.M111.338285 Contact: R. Luise Krauth-Siegel, Heidelberg University, Heidelberg, Germany e-mail: luise.krauth-siegel@bzh.uni-heidelberg.de
Viral infection	IL-33 (NF-HEV)	Mouse studies suggest IL-33 could help treat viral infections. Mice expressing an IL-33 decoy receptor or lacking the IL-33 receptor had weaker Cd8 ⁺ T cell responses to viral infections than animals with normal IL-33 signaling. In wild-type mice, recombinant IL-33 increased vaccinia virus-induced Cd8 ⁺ T cell responses compared with saline. Next steps include additional studies in animal models and GMP production of recombinant IL-33. SciBX 5(7); doi:10.1038/scibx.2012.183 Published online Feb. 16, 2012	Unpatented; licensing status not applicable	Bonilla, W.V. <i>et al. Science</i> ; published online Feb. 9, 2012; doi:10.1126/science.1215418 Contact: Daniel D. Pinschewer, University of Geneva, Geneva, Switzerland e-mail: daniel.pinschewer@gmx.ch Contact: Max Löhning, Charité-University Medicine Berlin, Berlin, Germany e-mail: loehning@drfz.de
Neurology				
Neurology	Muscarinic acetylcholine receptor M2 (CHRM2; HM2)	An <i>in vitro</i> study determined the structure of HM2, which could be used to develop subtype-selective modulators of muscarinic receptors. Those receptors are potential targets in neurological diseases. A crystal structure of HM2 bound to an antagonist revealed an active site binding pocket that is conserved between subtypes and potential allosteric sites that are not conserved. Next steps could include designing ligands that bind in the allosteric sites and solving the structures of additional subtypes of muscarinic receptors. SciBX 5(7); doi:10.1038/scibx.2012.184 Published online Feb. 16, 2012	Unpatented; licensing status not applicable	Haga, K. <i>et al. Nature</i> ; published online Jan. 25, 2012; doi:10.1038/nature10753 Contact: Takuya Kobayashi, Kyoto University Faculty of Medicine, Kyoto, Japan e-mail: t-coba@mfour.med.kyoto-u.ac.jp Contact: Tatsuya Haga, Gakushuin University, Tokyo, Japan e-mail: tatsuya.haga@gakushuin.ac.jp Contact: Brian K. Kobilka, Stanford University School of Medicine, Stanford, Calif. e-mail: kobilka@stanford.edu

This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Other				
Drug-induced liver injury (DILI)	Endoplasmic reticulum to nucleus signaling 1 (ERN1; IRE1; IRE1 α); x-box binding protein 1 (XBP1)	Mouse studies suggest activating IRE1 α could help treat acetaminophen-induced liver toxicity. In mice, a liver-specific Xbp1 deficiency led to IRE1 α activation and subsequent increased resistance to acetaminophen-induced liver toxicity and injury compared with wild-type Xbp1 expression. Next steps could include identifying and evaluating compounds that activate IRE1 α in animal models. SciBX 5(7); doi:10.1038/scibx.2012.185 Published online Feb. 16, 2012	Patent and licensing status unavailable	Hur, K.Y. <i>et al. J. Exp. Med.</i> ; published online Jan. 30, 2012; doi:10.1084/jem.20111298 Contact: Ann-Hwee Lee, Harvard School of Public Health, Boston, Mass. e-mail: ahlee@hsph.harvard.edu Contact: Laurie H. Glimcher, same affiliation as above e-mail: lglimche@hsph.harvard.edu
Renal disease				
Renal damage	Activin receptor-like kinase 3 (ALK3)	<i>In vitro</i> and mouse studies suggest ALK3 agonists could help treat renal damage. In a mouse model of chronic renal injury, kidney tubule-specific knockout of Alk3 increased epithelial damage and fibrosis compared with normal ALK3 expression. In five mouse models of chronic and acute renal injury, the cyclic peptide-based ALK3 agonist THR-123 prevented progression or reversed fibrosis compared with no treatment. Thrasos Therapeutics Inc. hopes to start a Phase I trial of THR-123 to treat renal injury next year. SciBX 5(7); doi:10.1038/scibx.2012.186 Published online Feb. 16, 2012	Findings patented; available for licensing from Thrasos	Sugimoto, H. <i>et al. Nat. Med.</i> ; published online Feb. 5, 2012; doi:10.1038/nm.2629 Contact: Raghu Kalluri, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Mass. e-mail: rkalluri@bidmc.harvard.edu
Various				
Breast cancer; endometriosis	Hydroxysteroid 17- β dehydrogenase 1 (HSD17B1)	<i>In vitro</i> and cell culture studies identified HSD17B1 inhibitors that could treat estrogen-dependent diseases like breast cancer and endometriosis. Inhibiting HSD17B1, which catalyzes the synthesis of estradiol, is known to decrease hormone-dependent breast tumor growth in animals. <i>In vitro</i> , two hydroxybenzothiazole-based compounds selectively inhibited human HSD17B1 with nanomolar IC ₅₀ values. Ongoing studies include optimizing the compounds for testing in marmoset models of endometriosis. SciBX 5(7); doi:10.1038/scibx.2012.187 Published online Feb. 16, 2012	Patent application filed; available for licensing	Spadaro, A. <i>et al. J. Med. Chem.</i> ; published online Jan. 26, 2012; doi:10.1021/jm201711b Contact: Rolf W. Hartmann, Saarland University, Saarbruecken, Germany e-mail: rwh@mx.uni-saarland.de or rolf.hartmann@helmholtz-hzi.de

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
Chemistry			
Continuous flow method for synthesizing artemisinin from a dihydroartemisinic acid	A continuous flow synthesis method to produce artemisinin from a precursor molecule could be used to generate large quantities of the malaria drug. Dihydroartemisinic acid was converted to artemisinin by photochemically inducing oxidation with singlet oxygen, followed by acid-mediated bond cleavage and subsequent oxidation with triplet oxygen in a continuous flow reactor. The method could produce 200 g of artemisinin per day per reactor. Next steps at an undisclosed new company include commercializing the process and setting up collaborations with producers of the precursor molecules. Amyris Inc., the Institute for OneWorld Health and Sanofi have partnered to develop and produce semisynthetic artemisinin.	Patent application filed; licensed to an undisclosed new company	Lévesque, F. & Seeberger, P.H. <i>Angew. Chem. Intl. Ed.</i> ; published online Jan. 16, 2012; doi:10.1002/anie.201107446 Contact: Peter Seeberger, Max-Planck Institute of Colloids and Interfaces, Potsdam, Germany e-mail: peter.seeberger@mpikg.mpg.de
SciBX 5(7); doi:10.1038/scibx.2012.188 Published online Feb. 16, 2012			
Disease models			
Human stem cell-derived models of cortical networks	Human stem cell-derived models of cortical networks could aid the development of new treatments for neurological diseases. Human stem cells were differentiated into a population of cortical stem and progenitor cells and underwent an extended period of cortical neurogenesis and neuronal terminal differentiation. The resulting neurons acquired mature electrophysiological properties and formed functional excitatory synapses. Next steps include using the approach to model a cortical disease, such as Alzheimer's disease.	Patent application filed covering use in undisclosed indications; available for licensing from Cambridge Enterprise Ltd., the technology transfer arm of the University of Cambridge	Shi, Y. <i>et al. Nat. Neurosci.</i> ; published online Feb. 5, 2012; doi:10.1038/nn.3041 Contact: Frederick J. Livesey, University of Cambridge, Cambridge, U.K. e-mail: rick@gurdon.cam.ac.uk
SciBX 5(7); doi:10.1038/scibx.2012.189 Published online Feb. 16, 2012			
Induced pluripotent stem (iPS) cell-based model of HCV infection	iPS cells could be used to model the response of individual patients to HCV infection. Undifferentiated iPS cells were cultured in conditions to induce the production of liver-specific markers and hepatocyte-specific morphology. Hepatocyte-like cells derived from iPS cells supported HCV replication and developed an inflammatory response to infection. In the cells, Incivek telaprevir or an HCV NS5B polymerase inhibitor decreased viral replication compared with vehicle. Next steps include using iPS cell-derived hepatocytes from patients with distinct genetic backgrounds to understand how they respond differently to HCV infection and therapeutics. Incivek is a small molecule HCV NS3 protease inhibitor marketed by Vertex Pharmaceuticals Inc. and Johnson & Johnson to treat HCV infection.	Patent application filed; available for licensing	Schwartz, R.E. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Jan. 30, 2012; doi:10.1073/pnas.1121400109 Contact: Sangeeta N. Bhatia, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: sbhatia@mit.edu Contact: Charles M. Rice, The Rockefeller University, New York, N.Y. e-mail: ricec@rockefeller.edu
SciBX 5(7); doi:10.1038/scibx.2012.190 Published online Feb. 16, 2012			
Induced pluripotent stem (iPS) cell-derived neurons from patients with Alzheimer's disease (AD)	<i>In vitro</i> studies suggest differentiating neurons from patient-derived iPS cells could help determine the mechanisms of sporadic AD. Neurons differentiated from fibroblast-derived iPS cells from two patients with familial AD and one patient with sporadic AD displayed disease pathology markers, including higher levels of secreted β -amyloid 40 (A β 40), greater amounts of phosphorylated- τ (p- τ) 231 and active glycogen synthase kinase 3 β (GSK3B), whereas neurons from healthy controls and one patient with sporadic AD did not. Next steps could include generating iPS cell-derived neurons from a larger population of patients with sporadic AD.	Patent and licensing status unavailable	Israel, M.A. <i>et al. Nature</i> ; published online Jan. 25, 2012; doi:10.1038/nature10821 Contact: Lawrence S.B. Goldstein, University of California, San Diego, La Jolla, Calif. e-mail: lgoldstein@ucsd.edu
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