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Crafting cancer combinations

By *Joanne Kotz, Senior Editor*

Two U.S. groups have developed platforms that could improve the identification of cancer drug combinations that address drug resistance. A team from **The University of North Carolina at Chapel Hill School of Medicine** is using chemical proteomics to rationally design kinase inhibitor combinations that block signaling pathways mediating drug resistance,¹ whereas **Massachusetts Institute of Technology** researchers are screening for targeted therapeutics that sensitize cancer cells to DNA-damaging agents when given sequentially rather than simultaneously.²

Each group has provided proof of principle for their platform by identifying a combination that showed efficacy in a mouse model of triple-negative breast cancer (TNBC).

Previous efforts to develop drug combinations that counter drug resistance in cancer have often focused on targeting genetic alterations in tumors that are acquired or become more prevalent in response to therapy. However, resistance can also be mediated by drug-induced changes in signaling pathways.

“Drug resistance is probably the biggest challenge that we face in cancer therapeutics today. I think the most important lesson from these two papers is that tumor cells can have an extraordinarily complex adaptive rewiring response to drug treatment, and we need to understand these responses at the protein network level, in addition to at the genetic level, in order to predict outcomes and select the best combination treatments,” said Paul Workman, deputy CEO and director of the **Cancer Research UK** Cancer Therapeutics Unit at **The Institute of Cancer Research**.

Kinase to kinome

A team led by Gary Johnson set out to understand how kinase-signaling pathways in tumors respond to kinase inhibitors, with the hope that the information might guide the design of combination treatments. Johnson is chair of the Department of Pharmacology at the UNC School of Medicine.

The UNC researchers focused on TNBC, a subtype defined by the lack of three markers: estrogen receptor and progesterone receptor expression and HER2 (EGFR2; ERBB2; neu) amplification. There are no targeted therapies approved for TNBC, and chemotherapy is standard of care.

In tumor tissue from a patient with TNBC, more than 400 of the 518 human kinases were expressed. Of the 400 expressed kinases, about half could be profiled using chemical proteomics—a technique in which



EDITORIAL**Editor-in-Chief:** Karen Bernstein, Ph.D.**Managing Editor:** Gaspar Taroncher-Oldenburg, Ph.D.**Executive Editor:** Steve Edelson**Senior Editors:** Tracey Baas, Ph.D.; Joanne Kotz, Ph.D.**Writers:** Chris Cain, Ph.D.; Michael Flanagan;

Tim Fulmer, Ph.D.; Michael J. Haas; Stephen Hansen; Kai-Jye Lou;

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Research Director: Walter Yang**Research Manager:** Kevin Lehnbeuter**Production Editors:** Brandy Cafarella; Carol Evangelista**Copy Editor:** Nicole DeGennaro**Editorial Assistant:** Mark Zipkin**Design:** Claudia Bentley; Miles DaviesFor inquiries, contact editorial@scibx.com**PUBLISHING****Publisher:** Peter Collins, Ph.D.**Associate Publishers:** Gaspar Taroncher-Oldenburg, Ph.D.; Eric Pierce**Marketing:** Sara Girard; Rosy Rogers**Technology:** Anthony Barrera; Julia Kulikova**Sales:** Ron Rabinowitz; Dean Sanderson; Tim Tulloch**OFFICES****BioCentury Publications, Inc.**

San Francisco

PO Box 1246

San Carlos, CA 94070-1246

T: +1 650 595 5333

Chadds Ford

223 Wilmington-West Chester Pike

Chadds Ford, PA 19317

T: +1 610 558 1873

Chicago

20 N. Wacker Drive, Suite 1465

Chicago, IL 60606-2902

T: +1 312 755 0798

Oxford

287 Banbury Road

Oxford OX4 7JA

United Kingdom

T: +44 (0)18 6551 2184

Washington, DC

2008 Q Street, NW, Suite 100

Washington, DC 20009

T: +1 202 462 9582

Nature Publishing Group

New York

75 Varick Street, 9th Floor

New York, NY 10013-1917

T: +1 212 726 9200

London

The Macmillan Building

4 Crinan Street

London N1 9XW

United Kingdom

T: +44 (0)20 7833 4000

Tokyo

Chiyoda Building 6F

2-37 Ichigayatamachi

Shinjuku-ku, Tokyo 162-0843

Japan

T: +81 3 3267 8751

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mass spectrometry is used to quantitatively measure the activation state of kinases.

The team found that tumor tissue had greater activation of the RAF-MEK-ERK pathway than adjacent normal mammary tissue. In human TNBC cell lines, the MEK inhibitor selumetinib initially blocked signaling downstream of MEK and blocked cell growth. However, after 24 hours of treatment, downstream signaling was reactivated and the tumor cells began to grow again.

Array BioPharma Inc. and **AstraZeneca plc's** selumetinib (AZD6244) is in Phase II testing in solid tumors.

Johnson's team hypothesized that kinase inhibitors against MEK could be activating other kinase-signaling pathways, thus blunting long-term efficacy. To find out if this is the case, the group used its platform to profile changes in kinase activation in tumors in response to selumetinib in a genetically engineered mouse model of TNBC.

In the tumors, giving selumetinib led to the upregulation of at least 30 kinases. The upregulated kinases included known targets of the multitargeted kinase inhibitor Nexavar sorafenib. Indeed, treating the TNBC mice with selumetinib plus Nexavar prevented the upregulation of about two-thirds of these kinases.

Onyx Pharmaceuticals Inc. and **Bayer AG** market Nexavar for liver and renal cancers. The drug is in Phase III testing in combination with chemotherapy in relapsed or refractory HER2-negative breast cancer.

Blocking the selumetinib-induced kinase upregulation resulted in a therapeutic benefit. In TNBC mice, selumetinib plus Nexavar led to tumor regression in 77% of the animals compared with 30% for selumetinib alone and 0% for Nexavar alone.

Results were published in *Cell*.

Building on the results in the mouse model, the UNC team is now using chemical proteomics to look at the effects of the **GlaxoSmithKline plc** MEK inhibitor trametinib (GSK1120212) on kinome activation in tumors from patients with TNBC. Trametinib inhibits MAP kinase kinase 1 (MAP2K1; MEK1) and MEK2 (MAP2K2).

In January, the researchers launched a trial to monitor the activity of kinases in tumor tissue before and after one week of treatment with trametinib. The trial is enrolling 10 patients and is expected to be completed by November. The trial is investigator sponsored, and the drug is not being dosed long enough to assess efficacy.

This month, GSK announced positive Phase III results with trametinib in metastatic melanoma patients with activating BRAF mutations. The molecule also is in Phase II testing to treat pancreatic cancer and relapsed or refractory solid tumors. Trametinib has not been tested in patients with TNBC.

Data linking the targeted inhibition of oncogenic pathways with the reprogramming of key kinases "suggests that the most effective approach to treating an individual's tumor may not only depend on the hardwired genetics but also on how each tumor changes at the molecular level after dosing with novel therapies," said Kiran Patel, development leader for GSK's MEK program.

"The most effective approach to treating an individual's tumor may not only depend on the hardwired genetics but also on how each tumor changes at the molecular level after dosing with novel therapies."
—Kiran Patel, GlaxoSmithKline plc

“It is largely unknown how predictable or variable this reprogramming may be between different patients,” said Patel. The UNC trial “will give us a more complete understanding of the potential diversity of kinase modulation by trametinib in triple-negative breast cancers, which could lead to more effective combination strategies for this tumor type in the future.”

Johnson told *SciBX* the UNC team is planning to use the same proteomic approach to look at the kinome response to HER2-directed antibodies and small molecules in breast cancer and to targeted therapeutics in leukemia and pancreatic and renal cancers.

A patent application was filed on the technology and is available for licensing, Johnson said.

Drug combo two-step

An MIT group led by Michael Yaffe took a different tack to identify drug combinations that target signaling pathways: looking for drugs that were synergistic when dosed sequentially.

Yaffe is a professor in the Department of Biology.

Because some patients with TNBC respond to DNA-damaging chemotherapeutics, the researchers reasoned it might be possible to identify a targeted therapeutic that, if given in advance of chemotherapy, could rewire the signaling pathways of tumor cells to increase their sensitivity to DNA-damaging agents.

To look for such an effect, the MIT team screened combinations of targeted therapeutics and DNA-damaging agents in a TNBC cell line. In the screen, the combination of drugs—and the order in which they were dosed—was varied.

Consistent with previous results, the researchers found that treating TNBC cells with Tarceva erlotinib alone or coadministration of Tarceva and doxorubicin did not potently induce apoptosis. However, treating the cells with Tarceva at least four hours before doxorubicin induced activation of caspase-8 (CASP8; FLICE), a key mediator of apoptosis, and increased cell killing as much as fivefold over simultaneous treatment with the two drugs ($p < 0.0001$).

Astellas Pharma Inc., Chugai Pharmaceutical Co. Ltd. and **Roche** market Tarceva, an epidermal growth factor receptor 1 (EGFR1; HER1; ERBB1) inhibitor, for pancreatic cancer and non-small cell lung cancer (NSCLC). The drug also is in Phase III testing for liver cancer.

The team also looked at the effect of the sequential combination in a panel of TNBC cell lines. Tarceva followed by doxorubicin induced synergistic cell killing in 4 of 10 TNBC cell lines tested. Cell line sensitivity correlated with basal EGFR activation—determined by the level of phosphorylated EGFR—not EGFR expression.

This result suggests the activity of the signaling pathway, rather than genetic alterations or gene expression, is important for determining drug response.

Finally, the team looked at the effects of the drug combination *in vivo*. In a xenograft mouse model of TNBC, doxorubicin alone, coadministration of Tarceva and doxorubicin, and sequential administration of Tarceva and doxorubicin all initially led to tumor regression. However, only the sequential combination prevented tumor regrowth.

Data were published in *Cell*.

“This work introduces a novel paradigm for combination treatments in oncology. What is particularly appealing is that the researchers

could show that EGFR phosphorylation, not *EGFR* gene amplification, is predictive of the sensitizing effect of sequential erlotinib and doxorubicin treatment in the small sample set of cell lines tested,” said Birgit Schoeberl, VP of discovery at cancer company **Merrimack Pharmaceuticals Inc.**

“I believe that this could be an interesting hypothesis to be tested clinically, especially since there are currently only limited treatment options for TNBC patients,” she added.

However, Schoeberl noted that identifying TNBC patients with activated EGFR and optimizing a sequential dosing regimen could prove challenging.

“Since it is difficult to measure protein phosphorylation in tumor biopsies, the testing of EGFR phosphorylation as a potential predictive marker may be challenging clinically. Also, I think we would want to further understand how long a patient needs to be on anti-EGFR therapy prior to being treated with doxorubicin in order to obtain the maximal sensitizing effect to the subsequent chemotherapy,” she said.

Yaffe, who is a member of Merrimack’s scientific advisory board, told *SciBX* his team is working with a clinician at the **Dana-Farber Cancer Institute** to design a clinical trial of the sequential treatment regimen of Tarceva and doxorubicin.

A specific start date for the trial has not been determined.

The MIT team is now working on developing assays to look at EGFR activation in tumor biopsies and is looking for sequential treatments with potential efficacy in additional cancer indications, including pancreatic and lung cancers.

A patent application has been filed covering the technology, and the IP is available for licensing, Yaffe said.

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e-mail: glj@med.unc.edu
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Contact: Michael B. Yaffe, Massachusetts Institute of Technology, Cambridge, Mass.
e-mail: myaffe@mit.edu

COMPANIES AND INSTITUTIONS MENTIONED

Array BioPharma Inc. (NASDAQ:ARRY), Boulder, Colo.
Astellas Pharma Inc. (Tokyo:4503), Tokyo, Japan
AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Bayer AG (Xetra:BAYN), Leverkusen, Germany
Cancer Research UK, London, U.K.
Chugai Pharmaceutical Co. Ltd. (Tokyo:4519), Tokyo, Japan
Dana-Farber Cancer Institute, Boston, Mass.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
The Institute of Cancer Research, Sutton, U.K.
Massachusetts Institute of Technology, Cambridge, Mass.
Merrimack Pharmaceuticals Inc. (NASDAQ:MACK), Cambridge, Mass.
Onyx Pharmaceuticals Inc. (NASDAQ:ONXX), South San Francisco, Calif.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
The University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, N.C.

β testing adenosine receptor agonists

By Tim Fulmer, Senior Writer

Researchers at the **University of California, San Francisco** have used a zebrafish screen to identify an adenosine receptor agonist that improved both β cell regeneration and glucose control in diabetic mice.¹ The discovery could open up a new indication for a class of compounds that until now have been developed mainly for inflammatory and cardiovascular disorders.

Despite efforts to discover small molecules that trigger regeneration of insulin-secreting β cells, the compounds have eluded diabetes drug developers because *in vitro* screens generally cannot reflect the complex, interrelated pathways and multiple types of progenitor cells that take part in β cell development.

Thus, a UCSF team led by Didier Stainier and Olov Andersson turned to a phenotype-based *in vivo* screen. “It allows for the discovery of compounds that target various cellular mechanisms of regeneration, which is important because regeneration of β cells can potentially occur through several mechanisms,” Andersson told *SciBX*.

Stainier is professor of biochemistry and biophysics at UCSF. Andersson has been a postdoctoral research fellow in Stainier’s lab since 2008 and is transitioning to an assistant professor post at the **Karolinska Institute**.

In prior work, Stainier and colleagues had designed a zebrafish system that allowed for selective ablation of cell types to study their effects on zebrafish development and regeneration.² They adapted that system to selectively ablate β cells.

In zebrafish embryos with ablated β cells, the researchers screened about 7,000 small molecules for their ability to double the number of β cells after only 2 days of regeneration. Five compounds reached that threshold, of which four targeted adenosine signaling and adenosine metabolism.

The most potent was 5'-N-ethylcarboxamidoadenosine (NECA), which significantly increased β cell regeneration compared with vehicle control ($p=0.0002$). In the same zebrafish embryos, NECA restored normal glucose levels significantly faster than vehicle at both two and three days post-treatment ($p=0.0031$ and $p=0.0292$, respectively).

Additional mechanistic studies showed that the effects of NECA were mediated by the adenosine A_{2A} receptor, a zebrafish homolog of the adenosine A_{2A} receptor (ADORA_{2A}) in humans.

NECA had similar effects in mammals. In mouse islet cells, the molecule significantly increased β cell proliferation compared with vehicle ($p<0.01$). In a mouse model of chemically-induced diabetes, NECA also improved both β cell proliferation ($p=0.0019$) and glucose control ($p<0.001$).

The findings were published in *Cell Metabolism*.

Getting more selective

Despite the strong mouse data, NECA is ill suited as a drug candidate because it nonselectively agonizes all adenosine receptors, not just ADORA_{2A}.

“A selective A_{2A} agonist should thus be evaluated,” said Joel Linden, professor of inflammation biology at the **La Jolla Institute for Allergy & Immunology**. Linden and colleagues have shown that selectively agonizing ADORA_{2A} has anti-inflammatory effects in several mouse and cell culture models, including pulmonary inflammation,³ graft-versus-host disease (GvHD)⁴ and bacterial gastritis.⁵

Linden also has shown that agonizing ADORA_{2B} contributes to insulin resistance in mice.⁶

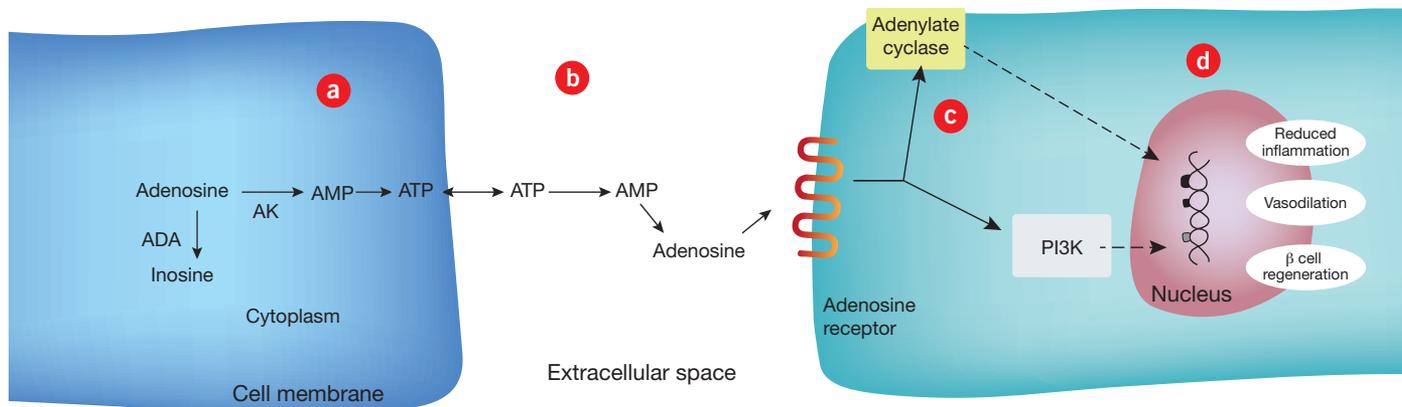


Figure 1. Agonizing β cell regeneration. A paper published by **University of California, San Francisco** researchers in *Cell Metabolism* suggests agonists of the adenosine A_{2A} receptor (ADORA_{2A}) can trigger regeneration of pancreatic β cells and thus could help treat diabetes. ADORA_{2A} plays a central role in adenosine metabolism and signaling.

Breakdown of adenosine by adenosine kinase (AK) and adenosine deaminase (ADA) generates essential metabolic molecules, including inosine, AMP and ATP [a]. When ATP is released into the extracellular space, it can be converted back to adenosine, which binds and activates ADORA_{2A} on the surface of neighboring cells [b]. ADORA_{2A} then triggers downstream signaling via G proteins and kinases, such as adenylate cyclase and phosphoinositide 3-kinase (PI3K) [c], which finally leads to expression of multiple genes involved in β cell regeneration, inflammation and vasodilation [d].

The UCSF group used a zebrafish screen to identify small molecules that acted on the adenosine pathway. The most potent compound, 5'-N-ethylcarboxamidoadenosine (NECA), agonized ADORA_{2A} and increased β cell proliferation in zebrafish and diabetic mice.

Andersson said he is studying adenosine agonists that are more specific than NECA in the zebrafish. Those include “A_{2A} receptor agonists that have been in clinical trials for other indications and have already gone through safety assessments,” he said. He declined to provide details about the compounds.

At least three ADORA_{2A} agonists have progressed into—or through—the clinic as vasodilators used as adjuncts to myocardial perfusion imaging (MPI) studies. These include Lexiscan regadenoson, which is marketed by **Gilead Sciences Inc.** and **Astellas Pharma Inc.**; **Pfizer Inc.**’s binodenoson, which is in registration; and **Forest Laboratories Inc.**’s apadenoson, which is in Phase III testing.

Given the broad effects even selective ADORA_{2A} agonists can have on cell types including immune and cardiac cells, it might be advisable to design ADORA_{2A} agonists that target the receptor specifically on β cells, according to Cord Dohrmann, CSO of **Evotec AG**.

Dohrmann declined to say how that might be accomplished. Published approaches to selectively target small molecules to β cells in animals have generally relied on peptides and peptide fragments.^{7,8}

In 2011, Evotec announced a deal with **Harvard University** and the **Howard Hughes Medical Institute** to discover and develop diabetes therapies targeting pancreatic β cell regeneration and replication. Earlier this year, Douglas Melton and colleagues published some of the first results of that collaboration in the *Proceedings of the National Academy of Sciences*.

Melton, a professor of molecular and cellular biology at Harvard and co-director of the **Harvard Stem Cell Institute**, used a screening platform based on freshly isolated rat islet cells to screen for small molecules that promoted replication of β cells. They identified a class of adenosine kinase inhibitors that promoted replication of cultured primary β cells from mice, rats and pigs.⁹

Adenosine kinase functions upstream of ADORA_{2A} in the adenosine signaling pathway (see Figure 1, “**Agonizing β cell regeneration**”). The adenosine kinase inhibitors are licensed to Evotec, Melton told *SciBX*. Dohrmann declined to disclose the company’s plans for the inhibitors.

Getting more human

Moving forward, the researchers will need to understand how the adenosine pathway works in human islets and whether long-term ADORA_{2A} agonism is safe in mammals.

Determining relevance of the adenosine signaling pathway to human β cell proliferation is a critical next question, Andrew Stewart told *SciBX*. Thus, he suggested testing ADORA_{2A} agonists in human fetal or neonatal cadaveric islets or, alternatively, in β cells derived from human induced pluripotent stem (iPS) cells or embryonic stem cells.

Stewart is professor of medicine at the **University of Pittsburgh School of Medicine** and chief of the Division of Endocrinology and Metabolism.

Stewart and colleagues at the **Broad Institute of MIT and Harvard** have developed a high throughput platform based on cultured human islets that they are using to screen for small molecule inducers of β cell proliferation.¹⁰

Stewart said a primary focus of his own work is developing ways to activate cell cycle proteins like cyclin dependent kinase 6 (CDK6), which has been shown to induce β cell proliferation in cultured human islets.^{11,12}

“From a safety standpoint, it will be critical to understand the effects of chronic stimulation of the A_{2A} receptor in rodents and monkeys,” said Hui Tian, VP and head of research at **NGM Biopharmaceuticals Inc.**

Earlier this year, NGM and **Daiichi Sankyo Co. Ltd.** partnered to discover and develop therapeutics that modulate β cell regeneration to treat diabetes. NGM is using its large-scale, high throughput, *in vivo* screening platform to discover and evaluate therapeutic candidates in rodents, said Tian.

“We are primarily focused on biologics—peptides, proteins and antibodies—since we believe they can achieve better target or cell specificity than small molecules,” he added.

Finally, Linden pointed out that islet regeneration by itself “does not solve the problem of autoimmunity and immune rejection of new islets.”

Andersson acknowledged that “there might be a need to combine a β cell–regenerative therapy with an immune suppressant” in type 1 diabetes, adding that “a β cell–regenerative therapy might also be advantageous for patients with late-stage type 2 diabetes who have reduced β cell mass and where immune responses are less prominent.”

The findings in the *Cell Metabolism* paper are not patented, Andersson said.

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Contact: Olov Andersson, University of California, San Francisco, Calif.
e-mail: olov.andersson@ki.se
Contact: Didier Y.R. Stainier, same affiliation as above
e-mail: didier.stainier@ucsf.edu
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COMPANIES AND INSTITUTIONS MENTIONED

Astellas Pharma Inc. (Tokyo:4503), Tokyo, Japan
Broad Institute of MIT and Harvard, Cambridge, Mass.
Daiichi Sankyo Co. Ltd. (Tokyo:4568; Osaka:4568), Tokyo, Japan
Evotec AG (Xetra:EVT), Hamburg, Germany
Forest Laboratories Inc. (NYSE:FRX), New York, N.Y.
Gilead Sciences Inc. (NASDAQ:GILD), Foster City, Calif.
Harvard Stem Cell Institute, Cambridge, Mass.
Harvard University, Cambridge, Mass.
Howard Hughes Medical Institute, Chevy Chase, Md.
Karolinska Institute, Stockholm, Sweden
La Jolla Institute for Allergy & Immunology, La Jolla, Calif.
NGM Biopharmaceuticals Inc., South San Francisco, Calif.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
University of California, San Francisco, Calif.
University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Vaccines revisited

By Tracey Baas, Senior Editor

A team from **Oregon Health & Science University** and **Najit Technologies Inc.** has shown that hydrogen peroxide could be a better way to inactivate viral vaccines than conventional methods such as formaldehyde and β -propiolactone.¹ Najit, which was spun out of the university in 2004, hopes to start a Phase I trial of an inactivated yellow fever virus vaccine in the next 1–2 years.

Noninfectious vaccines are typically produced by chemically inactivating a virus using formaldehyde or β -propiolactone. This approach can damage a virus so that it can no longer replicate, but the approach also can damage the antigenic epitopes required for an efficient immunological response.

Antibodies produced in response to the damaged epitopes may not efficiently interact with a live virus and neutralize it during future infection.

The Oregon team decided to try inactivating virus using a known antimicrobial and antiseptic agent: hydrogen peroxide. Conventional wisdom held that the oxidizing chemical would be too damaging to use for virus inactivation,² but the researchers thought otherwise.

First, the Oregon group showed that hydrogen peroxide inactivated the orthopoxviruses vaccinia virus and monkeypox virus, the flaviviruses West Nile virus (WNV) and yellow fever virus, and the arenavirus lymphocytic choriomeningitis virus (LCMV). In all cases, the result was undetectable levels of replicating virus.

In an ELISA, hydrogen peroxide–inactivated yellow fever virus retained higher levels of antigenicity against serum from yellow fever virus–infected mice than formaldehyde- or β -propiolactone-inactivated virus. This result suggests that hydrogen peroxide inactivation preserves more native antibody-binding sites.

The vaccine platform also provided protective immunity against chronic viral infection and lethal viral challenge.

In mice challenged with LCMV, those previously vaccinated with hydrogen peroxide–inactivated LCMV had a reduction in viral titers of more than 99% or had fully cleared the virus within 7 days, whereas unvaccinated mice remained viremic for at least 35 days.

Finally, mice vaccinated with hydrogen peroxide–inactivated vaccinia virus or WNV survived lethal viral challenge and had higher neutralizing antibody titers than

mice vaccinated with formaldehyde- or UV-inactivated viruses.

Data were published in *Nature Medicine*.

“We chose three unrelated virus model systems in order to demonstrate that we have a platform technology that can be used to prevent a wide range of diseases, including both chronic and lethal infection,” said Mark Slifka, professor and senior scientist at the Oregon

National Primate Research Center at OHSU and president and CSO at Najit.

One pathogen at a time

Najit thinks its hydrogen peroxide–inactivated vaccine platform—called HydroVax—will be amenable to producing vaccines against nearly any type of pathogen. The company’s initial focus is on viruses with limited vaccine options.

“We’re going to first focus our attention on a yellow fever virus vaccine,” said Ian Amanna, associate VP of Najit. “Despite the commercial availability of live attenuated yellow fever virus vaccines, they are contraindicated in infants and may place elderly patients at increased risk of severe adverse events.”

Novartis AG markets Arilvax and **Sanofi** markets YF-VAX, both live attenuated virus vaccines against yellow fever. Yellow fever vaccines in the clinic include **Johnson & Johnson’s** Flavimun, a live attenuated virus vaccine in Phase III testing, and **General Electric Co.’s** XR-001, a purified whole-virus, β -propiolactone-inactivated yellow fever vaccine adsorbed to an alum adjuvant. The vaccine is in Phase I trials.

“We are also interested in West Nile virus and dengue virus vaccines because there is currently no licensed vaccine available. However, there are live attenuated vaccines in various stages of clinical development,” said Amanna. “With live attenuated dengue virus vaccines, it has been somewhat challenging to get broad immunity to all four serotypes, but with an inactivated vaccine platform we have preliminary preclinical evidence indicating that this can be accomplished by adjusting the ratio of the different strains in the vaccine formulation.”

Further down the line, Slifka said the company is “interested in showing that our system also works for common viral pathogens such as influenza virus and respiratory syncytial virus.” Respiratory syncytial virus (RSV) vaccines of the past have been tricky. Formaldehyde-inactivated RSV vaccines have been shown to be well tolerated in children, but then exposure to natural RSV resulted in a number of hospitalizations and deaths, which has been attributed to exacerbated disease.

“We will continue to put our main focus on developing vaccines for diseases that already have well-established animal models,” he continued. “It may be awhile before we can test an RSV vaccine to determine if it provides protection or if it might exacerbate disease. It remains an interesting and open question.”

Slifka and Amanna also think the vaccine production technology could be used to inactivate bacteria, parasites and even bacterial spores.

“We have not initiated studies on nonviral targets but hope to begin these studies later this year,” Slifka said. “Who knows—it would be great to develop an effective vaccine for *Mycobacterium tuberculosis*. TB is a huge global problem, and current vaccine approaches have had only limited success.”

Ongoing work includes developing improved flavivirus vaccines and cGMP manufacturing of hydrogen peroxide–inactivated vaccines against yellow fever virus, WNV and dengue hemorrhagic fever.

The work is patented by OHSU and is licensed by Najit, which is looking for partners to co-develop or sublicense the technology for vaccine manufacture of specific targets.

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“We chose three unrelated virus model systems in order to demonstrate that we have a platform technology that can be used to prevent a wide range of diseases, including both chronic and lethal infection.”

—Mark Slifka,
Najit Technologies Inc.

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e-mail: slifkam@ohsu.edu
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COMPANIES AND INSTITUTIONS MENTIONED

General Electric Co. (NYSE:GE), Fairfield, Conn.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
Najit Technologies Inc., Beaverton, Ore.
Novartis AG (NYSE:NVS; SWX:NOVN), Basel, Switzerland
Oregon Health & Science University, Beaverton, Ore.
Sanofi (Euronext:SAN;NYSE:SNY), Paris, France



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Sequencing MRD

By Lauren Martz, Staff Writer

A group at the **Fred Hutchinson Cancer Research Center** has developed a high throughput sequencing platform to detect minimal residual disease in patients with T cell acute lymphoblastic leukemia that could be more accurate and cost effective than current approaches. The team has spun out **Adaptive Biotechnologies Corp.** to develop the technology.

Previous T-ALL studies have shown that minimal residual disease (MRD), or cancer cells that remain in circulation following treatment, can be a good indicator of patient outcomes and risk of relapse. Current strategies to detect MRD—flow cytometry and quantitative PCR—each measure the amount of cancer cells remaining in the blood or bone marrow after treatment, but both have limitations, according to Adaptive cofounder and Fred Hutchinson associate member Harlan Robins.

“There are four primary difficulties with using flow cytometry for measuring MRD. First, it has been difficult to standardize across different labs because there is not one established protocol. Second, the antibodies used to tag the cancerous cells are expensive. Third, every cancer type requires a different test because the protein markers vary for each disease. Finally, the sensitivity is low, which means that cancerous cells are sometimes not recognized,” he said.

Allele-specific quantitative PCR involves isolating and amplifying the cancer clone in each patient and is slow—taking more than a month to design, create and test an assay for each patient, he added.

His team opted for a third approach to detect MRD—relying on the often unique gene rearrangements that occur in T cell receptors (TCRs) as a result of the disease. Robins and colleagues reasoned that genotyping TCR loci could help diagnose T cell malignancies and that the genetic information obtained from high throughput sequencing of the regions could help determine whether cancer cells persist after treatment.

In 43 T-ALL samples taken at the time of diagnosis and before treatment, complete sequencing of the T cell receptor β -chain (TCRB) region detected specific gene rearrangements in 31 of the samples.

Using matched blood samples from the same patients 29 days after treatment, the team screened each sample for the patient-specific TCRB sequences to determine whether cancer cells were still present. The screening method identified 22 cases of MRD, including 10 that were not detected by conventional flow cytometry. In nine cases, neither method detected MRD.

Robins noted that the assays used in this paper limited the researchers to studying 200,000 cells. “We could certainly have sequenced more blood or bone marrow cells and found the rare clones in some of

the patients with no reported MRD,” he said. But “below the level of 1 cancerous cell in 100,000, no data exist on correlation with clinical outcome. So, if we tested for one cancer cell in a million, we would not have information on prognosis.”

He added that other versions of the company’s assay are capable of sequencing more than 10,000,000 cells.

Results were published in *Science Translational Medicine*.¹

“The next steps to move this strategy towards the clinic are to broaden the research to a larger patient population, follow the patients for a longer period of time to demonstrate a correlation between the detection of MRD and survival and expedite the run time on sequencing machines from weeks to days,” said Robins.

He added that Adaptive already is running studies on cells from many more patients with a variety of T and B cell cancers. The company is involved with at least 4 trials that will enroll a total of 500–1,000 patients each.

The company hopes to have its lab CLIA certified by year end and to have its MRD assay, dubbed immunoSEQ, available for clinical use within a year.

Measuring MRD is the first of several potential clinical uses of high throughput immune sequencing in oncology. ImmunoSEQ, which sequences T and B cell receptor chains, also is being used to segment patients according to likelihood of

response to cancer immunotherapeutics, said Robins.

“Beyond oncology, immunoSEQ is also being studied to identify and develop immunologic biomarkers in various autoimmune diseases such as multiple sclerosis, Crohn’s disease and lupus,” he added. “Adaptive is also developing a second assay platform, called quanTILfy, to measure the quantity and activity of immune cells in solid tumors.”

Adaptive’s quanTILfy is a platform to sequence and quantify tumor-infiltrating lymphocytes (TILs).

Adaptive has filed patent applications and licensed patents from Fred Hutchinson covering the TCR sequencing platform. The IP is not available for licensing.

Martz, L. *SciBX* 5(24); doi:10.1038/scibx.2012.620
Published online June 14, 2012

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Contact: Harlan Robins, Fred Hutchinson Cancer Research Center, Seattle, Wash.
e-mail: hrobins@fhcrc.org
Contact: Brent L. Wood, University of Washington, Seattle, Wash.
e-mail: woodbl@u.washington.edu

COMPANIES AND INSTITUTIONS MENTIONED

Adaptive Biotechnologies Corp., Seattle, Wash.
Fred Hutchinson Cancer Research Center, Seattle, Wash.

“The next steps to move this strategy towards the clinic are to broaden the research to a larger patient population, follow the patients for a longer period of time to demonstrate a correlation between the detection of MRD and survival and expedite the run time on sequencing machines from weeks to days.”

**—Harlan Robins,
Fred Hutchinson Cancer Research Center**

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				
Lupus; rheumatoid arthritis (RA); multiple sclerosis (MS)	MicroRNA-23b (miR-23b)	Patient sample and mouse studies suggest agonizing miR-23b could help treat autoimmune diseases such as lupus, RA and MS. In patients with RA or systemic lupus erythematosus (SLE), diseased tissues showed less miR-23b than healthy tissues. In mouse models of RA, MS and lupus, overexpression of miR-23b decreased both expression of inflammatory genes and disease progression compared with normal miR-23b expression. Next steps include developing a miR-23b agonist to treat autoimmune diseases. SciBX 5(24); doi:10.1038/scibx.2012.621 Published online June 14, 2012	Patent application filed; available for licensing	Zhu, S. <i>et al. Nat. Med.</i> ; published online June 3, 2012; doi:10.1038/nm.2815 Contact: Youcun Qian, Shanghai Institutes for Biological Sciences, Shanghai, China e-mail: ycqian@sibs.ac.cn
Cancer				
Brain cancer	Inducible nitric oxide synthase 2 (NOS2; iNOS); epidermal growth factor receptor variant III (EGFRvIII)	<i>In vitro</i> and mouse studies suggest iNOS inhibitors could help treat EGFRvIII-positive gliomas. An EgfrvIII-positive murine astrocyte cell line showed higher levels of iNOS and greater proliferation and invasiveness than EgfrvIII-negative controls. In normal mice injected with EgfrvIII-positive astrocytes, iNOS knockdown resulted in smaller, less invasive tumors than no knockdown. In mice with subcutaneous EgfrvIII-positive tumors, an iNOS inhibitor decreased tumorigenesis and tumor growth compared with vehicle. Next steps could include identifying additional molecular targets involved in iNOS-mediated glioma growth and invasion. Rindopepimut (CDX-110; ALT-110), a vaccine targeting EGFRvIII from Celldex Therapeutics Inc., is in Phase III testing to treat EGFRvIII-positive glioblastoma and other brain tumors. CR 3294, an iNOS and matrix metalloproteinase (MMP) co-inhibitor from EpiStem Holdings plc and Rottapharm Madaus, is in Phase II testing to treat mucositis. nNOS/iNOS, a selective neuronal nitric oxide synthase 1 (NOS1; nNOS) and iNOS inhibitor from NeurAxon Inc., is in preclinical testing to treat pain. SciBX 5(24); doi:10.1038/scibx.2012.622 Published online June 14, 2012	Patent and licensing status unavailable	Puram, S.V. <i>et al. J. Neurosci.</i> ; published online June 6, 2012; doi:10.1523/JNEUROSCI.3243-11.2012 Contact: Azad Bonni, Harvard Medical School, Boston, Mass. e-mail: azad_bonni@hms.harvard.edu
Brain cancer	Unknown	Cell culture studies suggest the lipid 2-hydroxyoleate (2OHOA) could help treat glioma. In cultured human glioma cells, 2OHOA inhibited multiple proliferative signaling pathways and decreased growth compared with a control chemotherapeutic. Next steps include identifying the mechanism of action of 2OHOA and testing the compound in cell culture models of lung cancer. Lipopharma Therapeutics S.L.'s Minerval, a formulation of 2OHOA, is in preclinical development for glioma. SciBX 5(24); doi:10.1038/scibx.2012.623 Published online June 14, 2012	Patented; licensed to Lipopharma Therapeutics	Terés, S. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online May 14, 2012; doi:10.1073/pnas.1118349109 Contact: Pablo V. Escribá, University of the Balearic Islands, Palma de Mallorca, Spain e-mail: pablo.escriba@uib.es

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer	Dopamine receptor	<p>Patient sample and cell culture studies suggest the dopamine receptor antagonist thioridazine could help treat cancer. In samples from five patients with acute myelogenous leukemia (AML), thioridazine decreased proliferation and AML blast numbers compared with vehicle. Next steps could include determining the effects of thioridazine in an <i>in vivo</i> model of AML and in other cancers.</p> <p>Thioridazine is a generic first-generation antipsychotic.</p> <p>SciBX 5(24); doi:10.1038/scibx.2012.624 Published online June 14, 2012</p>	Patent and licensing status unavailable	<p>Sachlos, E. <i>et al. Cell</i>; published online May 24, 2012; doi:10.1016/j.cell.2012.03.049 Contact: Mickie Bhatia, McMaster University, Hamilton, Ontario, Canada e-mail: mbhatia@mcmaster.ca</p>
Cancer	Proline dehydrogenase 1 (PRODH; POX)	<p>Mouse and cell culture studies suggest inhibiting PRODH could help treat hypoxia-driven tumors. In a mouse xenograft model of human breast cancer, tumor regions with more hypoxia had higher PRODH levels than less hypoxic regions. In a human colorectal cancer cell line cultured under hypoxic conditions, a PRODH inhibitor and a PRODH-targeted small interfering RNA decreased cell viability compared with control siRNA. Next steps include evaluating the metabolic and therapeutic implications of proline metabolism pathways in additional cancer settings.</p> <p>SciBX 5(24); doi:10.1038/scibx.2012.625 Published online June 14, 2012</p>	Patent and licensing status undisclosed	<p>Liu, W. <i>et al. Cancer Res.</i>; published online May 18, 2012; doi:10.1158/0008-5472.CAN-12-0080 Contact: James M. Phang, National Cancer Institute, Frederick, Md. e-mail: phangj@mail.nih.gov</p>
Kaposi's sarcoma (KS)	EPH receptor A2 (EPHA2)	<p><i>In vitro</i> studies suggest EPHA2 could help prevent KS caused by KS-associated herpes virus (KSHV) infection. Coimmunoprecipitation studies showed that the KSHV glycoprotein dimer, which is required for cell entry, precipitates with EPHA2, suggesting it acts as the cell surface receptor for the virus. In human and mouse endothelial cells, antibodies or siRNA against EPHA2 protected against KSHV infection compared with control IgG or nontargeting siRNA. Next steps could include testing an anti-EPHA2 antibody in animal models of KSHV infection.</p> <p>SciBX 5(24); doi:10.1038/scibx.2012.626 Published online June 14, 2012</p>	Patent and licensing status unavailable	<p>Hahn, A.S. <i>et al. Nat. Med.</i>; published online May 27, 2012; doi:10.1038/nm.2805 Contact: Frank Neipel, University Hospital Erlangen, Erlangen, Germany e-mail: frank.neipel@viro.med.uni-erlangen.de</p>
Leukemia	Leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2; LIR2)	<p>Mouse and cell culture studies suggest inhibiting the interaction between angiopoietin-like proteins and LILRB2 could help treat leukemia. In a mouse model of acute myelogenous leukemia (AML), a deficiency in the signaling of the mouse ortholog of <i>LILRB2</i> increased survival times and differentiation of leukemia cells compared with no deficiency. In human and mouse cell cultures, angiopoietin-like proteins bound LILRB2 or the mouse ortholog and promoted the development of leukemia. Researchers did not disclose next steps, which could include screening for molecules that block the interaction.</p> <p>SciBX 5(24); doi:10.1038/scibx.2012.627 Published online June 14, 2012</p>	Patent application filed; licensing information available from the Office for Technology Development at The University of Texas Southwestern Medical Center	<p>Zheng, J. <i>et al. Nature</i>; published online May 30, 2012; doi:10.1038/nature11095 Contact: Cheng Cheng Zhang, The University of Texas Southwestern Medical Center, Dallas, Texas e-mail: alec.zhang@utsouthwestern.edu</p>

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Melanoma	c-Met proto-oncogene (MET; HGFR)	<i>In vitro</i> and mouse studies suggest inhibiting exosome secretion could help treat cancers including melanoma. In exosomes from highly metastatic melanomas, protein content and MET levels were greater than those in exosomes from less metastatic melanomas. In mouse xenograft models of human metastatic melanoma, injection of exosomes from metastatic melanomas increased bone and lung metastasis compared with injection of exosomes from less metastatic melanomas. In the xenograft mice, small hairpin RNA-mediated knockdown of <i>MET</i> decreased metastasis and tumor growth compared with normal <i>MET</i> expression. Next steps include evaluating the potential of the exosomes as diagnostic markers. SciBX 5(24); doi:10.1038/scibx.2012.628 Published online June 14, 2012	Patent applications filed by Weill Cornell Medical College; available for licensing	Peinado, H. <i>et al. Nat. Med.</i> ; published online May 27, 2012; doi:10.1038/nm.2753 Contact: David Lyden, Weill Cornell Medical College, New York, N.Y. e-mail: dcl2001@med.cornell.edu Contact: Jacqueline Bromberg, Memorial Sloan-Kettering Cancer Center, New York, N.Y. e-mail: bromberj@mskcc.org
Prostate cancer	Androgen receptor (AR)	<i>In vitro</i> studies identified 1-(3-(2-chlorophenoxy)propyl)1 <i>H</i> -indole-3-carbonitrile (CPIC) as an AR antagonist that could help treat prostate cancer. In a cell-based high throughput screen of 160,000 small molecules, CPIC was identified as a competitive AR antagonist that specifically inhibited AR-mediated transcription by blocking its binding to androgen-responsive genes. In prostate cancer cell lines, CPIC inhibited cell growth and anchorage-independent proliferation in cells expressing mutant or wild-type AR but did not alter cell growth in those that did not express AR. Next steps include studies in mouse xenograft models. At least 11 companies have AR antagonists in development stages ranging from preclinical to marketed to treat prostate cancer. SciBX 5(24); doi:10.1038/scibx.2012.629 Published online June 14, 2012	Patent application filed covering the CPIC family; available for licensing	Cherian, M.T. <i>et al. J. Biol. Chem.</i> ; published online May 15, 2012; doi:10.1074/jbc.M112.344671 Contact: David J. Shapiro, University of Illinois, Urbana, Ill. e-mail: djshapir@life.illinois.edu
Endocrine/metabolic disease				
Diabetes	Adenosine A _{2A} receptor (ADORA _{2A})	Zebrafish and mouse studies suggest adenosine receptor agonists could regenerate β cells and help treat type 1 diabetes. A screen of 7,000 compounds in a transgenic zebrafish model of β cell regeneration identified 5 molecules that increased β cell regeneration. The most potent compound, 5'-N-ethylcarboxamidoadenosine (NECA), activated ADORA _{2A} and significantly increased β cell proliferation in zebrafish compared with vehicle ($p=0.001$). In a mouse model of chemically induced diabetes, NECA increased β cell proliferation and decreased blood glucose levels compared with vehicle. Next steps include identifying and testing adenosine receptor agonists with better specificity than NECA. Lexiscan, a short-acting ADORA _{2A} agonist from Gilead Sciences Inc. and Astellas Pharma Inc., is marketed as a cardiovascular imaging agent. CorVue binodenoson for injection, a selective ADORA _{2A} agonist from Pfizer Inc., is under review as a cardiovascular imaging agent. At least seven other companies have ADORA _{2A} agonists in Phase III testing or earlier to treat various indications (see β testing adenosine receptor agonists, page 4). SciBX 5(24); doi:10.1038/scibx.2012.630 Published online June 14, 2012	Unpatented; licensing status not applicable	Andersson, O. <i>et al. Cell Metab.</i> ; published online May 17, 2012; doi:10.1016/j.cmet.2012.04.018 Contact: Olov Andersson, University of California, San Francisco, Calif. e-mail: olov.andersson@ki.se Contact: Didier Y.R. Stainier, same affiliation as above e-mail: didier.stainier@ucsf.edu

This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Diabetes	Apolipoprotein A-IV (APOA4)	<p>Mouse studies suggest APOA4 could help treat type 2 diabetes. In mice fed a high-fat diet, ApoA4 deficiency lowered insulin secretion and decreased glucose tolerance compared with what was seen in nondeficient controls. In diabetic mice, injection of APOA4 increased insulin secretion and decreased blood glucose compared with injection of saline. Next steps include testing APOA4 safety in animals and designing modified variants that have an extended half-life.</p> <p>SciBX 5(24); doi:10.1038/scibx.2012.631 Published online June 14, 2012</p>	Patent application filed; licensed to HealthCare Ventures LLC	<p>Wang, F. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online May 22, 2012; doi:10.1073/pnas.1201433109</p> <p>Contact: Patrick Tso, University of Cincinnati, Cincinnati, Ohio e-mail: tsopp@ucmail.uc.edu</p>
Gaucher's disease	Glucocerebrosidase (GBA; GCCase)	<p>Mouse and <i>in vitro</i> studies identified small molecule chaperones of GCCase that could help treat Gaucher's disease. In patient-derived dermal fibroblasts, the lead compound increased GCCase protein levels compared with vehicle. In mice, the compound distributed to the target organs affected by Gaucher's disease. Next steps include evaluating the small molecules in patient-derived macrophages, running long-term toxicity studies and doing GMP scale up.</p> <p>Elelyso taliglucerase alfa, a plant cell-expressed recombinant form of human GCCase from Protalix Biotherapeutics Inc. and Pfizer Inc., is approved to treat Gaucher's disease.</p> <p>Amicus Therapeutics Inc.'s afegostat (AT2101), an orally available small molecule chaperone that binds to GCCase, is in Phase II testing for Gaucher's disease. Amicus' AT3375, a next-generation small molecule GCCase chaperone, is in preclinical development for Gaucher's disease.</p> <p>SciBX 5(24); doi:10.1038/scibx.2012.632 Published online June 14, 2012</p>	Patent application filed covering use in Gaucher's and Parkinson's disease; available for licensing	<p>Patnaik, S. <i>et al. J. Med. Chem.</i>; published online May 30, 2012; doi:10.1021/jm300063b</p> <p>Contact: Juan J. Marugan, National Institutes of Health, Rockville, Md. e-mail: maruganj@mail.nih.gov</p>
Genitourinary disease				
Endometriosis	Nuclear receptor coactivator 1 (NCOA1; SRC1)	<p>Patient sample and mouse studies suggest inhibiting an SRC1 fragment could help treat endometriosis. Endometriotic tissue derived from patients and mouse models showed higher levels of a 70 kDa SRC1 fragment than matched normal endometrial tissue. Human endometrial cell lines expressing the fragment had increased markers of endometriosis, such as invasiveness, compared with cells expressing full-length SRC1. In <i>Src1</i>-deficient mouse models of endometriosis, endometriotic lesion volumes were over fivefold lower than those of lesions from nondeficient mice with endometriosis. Ongoing work includes identifying inhibitors of the SRC1 fragment.</p> <p>SciBX 5(24); doi:10.1038/scibx.2012.633 Published online June 14, 2012</p>	Unpatented; available for licensing or partnering	<p>Han, S.J. <i>et al. Nat. Med.</i>; published online June 3, 2012; doi:10.1038/nm.2826</p> <p>Contact: Bert W. O'Malley, Baylor College of Medicine, Houston, Texas e-mail: berto@bcm.tmc.edu</p>

This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Infectious disease				
Pneumococcus; meningitis	α -Glycerophosphate oxidase (glpO)	<p>Mouse and <i>in vitro</i> studies suggest vaccines against glpO could help prevent pneumococcal meningitis. In mice infected with a <i>glpO</i>-deficient strain of <i>Streptococcus pneumoniae</i>, meningitis severity and bacterial counts in the brain were lower than those in brains infected with <i>glpO</i>-expressing strains. In mice challenged by meningococcal bacteria, immunization with glpO led to longer survival than immunization with alum. Next steps include developing optimized vaccine formulations of glpO with other pneumococcal antigens.</p> <p>SciBX 5(24); doi:10.1038/scibx.2012.634 Published online June 14, 2012</p>	<p>Patent application filed; licensing information available from The University of Adelaide</p> <p>Contact: Timothy Spencer, Adelaide Research & Innovation, Adelaide, South Australia, Australia e-mail: timothy.spencer@adelaide.edu.au</p>	<p>Mahdi, L.K. <i>et al. J. Clin. Invest.</i>; published online May 24, 2012; doi:10.1172/JCI45850</p> <p>Contact: Abiodun D. Ogunniyi, Research Centre for Infectious Diseases, The University of Adelaide, Adelaide, South Australia, Australia e-mail: david.ogunniyi@adelaide.edu.au</p>
Neurology				
Alzheimer's disease (AD)	Cyclophilin A (CYPA; PPIA)	<p>Mouse studies suggest antagonizing PPIA could be useful for treating AD. In mice with the AD-associated E4 allele of apolipoprotein E (APOE), knocking out <i>Ppia</i> or treatment with the PPIA inhibitor cyclosporine A decreased blood brain barrier permeability and microvascular damage compared with no knockout or with vehicle treatment. Next steps could include testing the effect of <i>Ppia</i> deletion or inhibition in additional mouse models of AD.</p> <p>SciBX 5(24); doi:10.1038/scibx.2012.635 Published online June 14, 2012</p>	<p>Patent and licensing status undisclosed</p>	<p>Bell, R.D. <i>et al. Nature</i>; published online May 16, 2012; doi:10.1038/nature11087</p> <p>Contact: Berislav V. Zlokovic, University of Southern California, Los Angeles, Calif. e-mail: berislav.zlokovic@med.usc.edu</p>
Pain	Not applicable	<p>Mouse studies suggest transplantation of inhibitory neuronal precursors to the spinal cord could help treat neuropathic pain. In mice, inhibitory neuron precursors from the medial ganglionic eminence (MGE) of the embryonic brain were transplanted into adult spinal cords and developed into inhibitory γ-aminobutyric acid (GABA)-producing interneurons. In a mouse model of neuropathic pain, the cell transplants decreased sensitivity to painful mechanical stimulus compared with no transplant. Next steps include identifying ways to scale up isolation and culture of the MGE cells.</p> <p>Neurona Therapeutics Inc., which was founded by the study authors, has MGE cells in discovery-stage development for pain, schizophrenia and Parkinson's disease (PD).</p> <p>SciBX 5(24); doi:10.1038/scibx.2012.636 Published online June 14, 2012</p>	<p>Patent pending; available for licensing</p>	<p>Bráz, J.M. <i>et al. Neuron</i>; published online May 24, 2012; doi:10.1016/j.neuron.2012.02.033</p> <p>Contact: João M. Bráz, University of California, San Francisco, Calif. e-mail: bjoao@phy.ucsf.edu</p>

This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Various				
Dermatology; pulmonary fibrosis	Not applicable	<i>In vitro</i> and mouse studies suggest the C-terminal domain of endostatin could help treat and prevent organ fibrosis. In an <i>ex vivo</i> human skin model of dermal fibrosis, a peptide fragment from the C terminus of human endostatin prevented and reversed fibrosis and skin thickening better than the unmodified peptide. In mouse models of dermal and pulmonary fibrosis, the peptide prevented and reversed fibrosis induced by transforming growth factor- β (TGFB; TGF β) or the chemotherapeutic bleomycin. Next steps include pharmacokinetic and toxicity studies. Simcere Pharmaceutical Group markets Endu recombinant human endostatin in China to treat non-small cell lung cancer (NSCLC). Oxford BioMedica plc's EndoAngio-GT, a gene therapy delivering the <i>endostatin</i> gene, is in preclinical testing to treat cancer.	Patent application filed; available for licensing	Yamaguchi, Y. <i>et al. Sci. Transl. Med.</i> ; published online May 30, 2012; doi:10.1126/scitranslmed.3003421 Contact: Carol A. Feghali-Bostwick, University of Pittsburgh School of Medicine, Pittsburgh, Pa. e-mail: feghca@upmc.edu Contact: Yukie Yamaguchi, same affiliation as above e-mail: yui1783@yahoo.co.jp Contact: Adriana T. Larregina, same affiliation as above e-mail: adriana@pitt.edu
SciBX 5(24); doi:10.1038/scibx.2012.637 Published online June 14, 2012				



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This week in techniques

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

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

Approach	Summary	Licensing status	Publication and contact information
Assays & screens			
Cell microarrays to identify drug-resistance pathways	Cell microarrays could be used to identify proteins and pathways mediating drug resistance in cancer. The microarrays are comprised of glass slides coated with a matrix to promote localized cell adherence. The arrays are treated with individual lentiviruses expressing small hairpin RNAs or open reading frames and are then seeded with adherent cell lines. One set of microarrays was treated with lentiviruses expressing kinase open reading frames and seeded with a human melanoma cell line bearing the constitutively active V600E <i>BRAF</i> mutation. Incubating these microarrays with a <i>BRAF</i> inhibitor identified kinases that conferred resistance to the inhibitor. Next steps include expanding the profiling to additional cancer drugs and the cell types and assays that can be used in the screens. SciBX 5(24); doi:10.1038/scibx.2012.638 Published online June 14, 2012	Patent application filed covering technology and its applications, including in drug-resistance modifier screens; available for licensing through the Whitehead Institute for Biomedical Research and the Broad Institute of MIT and Harvard	Wood, K.C. <i>et al. Sci. Signal.</i> ; published online May 15, 2012; doi:10.1126/scisignal.2002612 Contact: David M. Sabatini, Whitehead Institute for Biomedical Research, Cambridge, Mass. e-mail: sabatini@wi.mit.edu Contact: Kris C. Wood, same affiliation as above e-mail: kcwood@alum.mit.edu
Drug delivery			
Nanoparticle-mediated delivery of wortmannin	A nanoparticle formulation of the pan-phosphoinositide 3-kinase (PI3K) inhibitor wortmannin could reduce the drug's hepatotoxicity. In mice, a lipid- and polyethylene glycol (PEG)-coated nanoparticle formulation of wortmannin plus radiation therapy caused tumor growth inhibition that was comparable to that for a control formulation of the compound. In mice, the nanoparticle-formulated wortmannin had about fourfold higher maximum tolerated dose and lower hepatotoxicity than the control formulation. Next steps include further optimizing the formulation and testing it as a stand-alone therapeutic. At least 15 companies have PI3K inhibitors in preclinical through Phase III testing for a range of inflammatory and cancer indications. SciBX 5(24); doi:10.1038/scibx.2012.639 Published online June 14, 2012	Patent pending; available for licensing	Karve, S. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online April 30, 2012; doi:10.1073/pnas.1120508109 Contact: Andrew Z. Wang, The University of North Carolina at Chapel Hill, Chapel Hill, N.C. e-mail: zawang@med.unc.edu
Drug platforms			
Deep sequencing for optimizing protein-based inhibitors of influenza A virus hemagglutinin	Deep sequencing could be useful for optimizing computationally designed protein-based inhibitors for influenza. Deep sequencing identified single-point mutants of two proteins that strongly bound influenza hemagglutinin. <i>In vitro</i> , the most potent mutant cross-reacted with all influenza group 1 hemagglutinin proteins and bound them with higher affinity than the wild-type protein. The mutant also neutralized seasonal and pandemic strains of H1N1 at nanomolar concentrations. Next steps include testing the identified protein variants in animal models. SciBX 5(24); doi:10.1038/scibx.2012.640 Published online June 14, 2012	Patent application filed for treatment of influenza infection; licensing status undisclosed	Whitehead, T.A. <i>et al. Nat. Biotechnol.</i> ; published online May 27, 2012; doi:10.1038/nbt.2214 Contact: David Baker, University of Washington, Seattle, Wash. e-mail: dabaker@u.washington.edu
Engineering IgG4 mutants with reduced heterogeneity and improved thermal stability	Engineered IgG4 mutants that have reduced heterogeneity and increased thermal stability could help improve the production of IgG4-based biologics. The IgG4 mutants were engineered to have a light chain-heavy chain disulfide bond arrangement similar to that of IgG1 molecules and an S241P mutation, which increases thermal stability. <i>In vitro</i> , the engineered IgG4 molecules showed better thermal stability and less product heterogeneity than wild-type IgG4. Next steps could include generating a stabilized IgG4-based therapeutic mAb. SciBX 5(24); doi:10.1038/scibx.2012.641 Published online June 14, 2012	Patent and licensing status unavailable	Peters, S.J. <i>et al. J. Biol. Cell</i> ; published online May 18, 2012; doi:10.1074/jbc.M112.369744 Contact: Shirley J. Peters, UCB Pharma Slough, Slough, U.K. e-mail: shirley.peters@ucb.com

This week in techniques

Approach	Summary	Licensing status	Publication and contact information
Low-density nanofiber scaffold to support cartilage repair	A low-density nanofiber scaffold could be useful for cartilage repair. The scaffold is a poly(vinyl alcohol)-methacrylate (PVA-MA) and PVA-MA/chondroitin sulfate composite of nanofibers arranged in a low-density 3D network. In rats with a surgically induced osteochondral defect, implantation of the scaffold at the defect site increased cartilage repair compared with no implantation. Next steps include evaluating the scaffold in large animal models. SciBX 5(24); doi:10.1038/scibx.2012.642 Published online June 14, 2012	Patent application filed; unlicensed	Coburn, J.M. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 4, 2012; doi:10.1073/pnas.1121605109 Contact: Jennifer H. Elisseeff, The Johns Hopkins University, Baltimore, Md. e-mail: jhe@jhu.edu
Synthetic staphylococcal enterotoxin B (SEB)-neutralizing antibodies to prevent toxic shock	Synthetic SEB-neutralizing antibodies could help protect against toxic shock syndrome. A screen of a synthetic phage display library of human antibodies identified SEB-neutralizing antibodies. In a mouse model of SEB-induced toxic shock, injection of the lead antibody candidate one hour after induction protected against lethal shock in all animals. Next steps include testing the candidate in monkeys and in an aerosol SEB intoxication model. SciBX 5(24); doi:10.1038/scibx.2012.643 Published online June 14, 2012	Provisional patent application filed covering the anti-SEB antibody for therapeutic and prophylactic applications; available for licensing	Karauzum, H. <i>et al. J. Biol. Chem.</i> ; published online May 29, 2012; doi:10.1074/jbc.M112.364075 Contact: M. Javad Aman, Integrated BioTherapeutics Inc., Gaithersburg, Md. e-mail: javad@integratedbiotherapeutics.com Contact: Sachdev S. Sidhu, University of Toronto, Toronto, Ontario, Canada e-mail: sachdev.sidhu@utoronto.ca

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