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A public-private effort, kick-started by a CRADA between the FDA and Novartis, has created a roadmap for one step in the process of moving a biomarker from discovery to regulatory acceptance. The pathway applies to the preclinical use of biomarkers, in this case using them to test candidate compounds for kidney toxicity.

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University of Southern California researchers have shown that short-term starvation boosts chemotherapy resistance in normal cells but not in tumors. The group is readying a human clinical trial to test the starvation response and has formed a company based upon the findings.

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Two of three studies reported in *Nature Medicine* found signs of Parkinson's disease pathology in a small fraction of transplanted dopaminergic cells—the first time researchers have reported on the long-term fate of these transplants. The next question to resolve is why the pathology occurred. The answer could lead to improvements in cell-based therapies and perhaps illuminate the mechanisms behind PD.

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By Steve Usdin, Washington Editor

Every week, academic scientists publish dozens of articles on biomarkers. Some results are strong enough to persuade drug companies to use certain biomarkers to guide development decisions. But only a handful of the ones that have been incorporated into marketing applications are accepted by regulators as evidence of efficacy or safety.

Moving biomarkers from academic journals to drug labels is a prerequisite for advancing personalized medicine and for enhancing the overall efficiency of drug development. However, identifying prospective markers has proven far simpler than getting them accepted by regulators.

Potentially useful toxicity tests based on biomarkers get stuck in regulatory purgatory in large part because there has not been a process for determining what kinds of data need to be assembled to convince regulators that the tests are reliable, sensitive and specific predictors of safety or efficacy.

Now, a public-private effort kick-started by a Cooperative Research and Development Agreement (CRADA) between the **Food and Drug Administration** and **Novartis AG** has created a roadmap for one step in the process of moving a biomarker from discovery to regulatory acceptance for clinical studies or labeling.

This first pathway applies to FDA qualification of biomarkers for preclinical use. In addition to mapping out the pathway, the CRADA applied it in order to qualify several biomarkers to detect nephrotoxicity, an area in which existing preclinical tests are unable to assess toxicity until kidney damage is irreversible.

The project subsequently was expanded and turned over to the Predictive Safety Testing Consortium (PSTC), a group of drug companies that is being managed by a nonprofit organization, the **Critical Path Institute** (C-Path). C-Path was founded in 2005 by the FDA, **SRI International** and the **University of Arizona**.¹

The PSTC consists of 15 pharmaceutical companies, **ClinXus**, which is a nonprofit organization that conducts biomarker R&D, and predictive toxicology company **Iconix Biosciences Inc.**, a unit of **Entelos Inc.** (See **Table 1**, “**Predictive Safety Testing Consortium members**.”)

About 180 scientists from industry are working on five PSTC projects. In addition to nephrotoxicity, which is the most advanced project, the consortium is working on biomarkers for hepatotoxicity, vascular injury, nongenotoxic carcinogenicity and myopathy.

The nephrotoxicity project was clearly aimed at picking low-hanging fruit—there is much room for improvement on measures of kidney



Science-Business eXchange

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

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damage, a number of candidate markers are readily at hand and testing them is relatively straightforward.

Although none of the PSTC's nephrotoxicity biomarkers is new—total urinary protein and albumin, for example, have been measured for decades—they cannot currently be used to support drug applications because the work necessary to qualify them for regulatory purposes had never been conducted.

“The CRADA with Novartis kick-started the effort that is going on now in the consortium and provided a blueprint for the pilot biomarker qualification process that we have been setting up at the agency,” Felix Frueh, associate director of genomics in the FDA's Office of Clinical Pharmacology and Biopharmaceutics, told *SciBX*.

New nephrotoxicity biomarkers are needed because the toxicity markers that regulators accept today don't produce signals until injury is irreversible. Frueh noted that a signal from blood urea nitrogen (BUN) or creatinine tests can effectively kill a compound.

“The current markers, BUN and serum creatinine, tell you how dead your kidney is, but are not very useful in assessing a gradual scale of damage,” he said.

The new toxicity markers would dramatically increase the sensitivity and specificity of preclinical tests for kidney damage, according to Frueh. This could give industry and regulators confidence to continue the development of compounds in the face of safety signals that, based on older, blunter instruments, would have been showstoppers.

Frueh said the ability to “detect toxicity very, very early, while it is reversible” would make it possible for the FDA to allow clinical research on compounds even if there is some evidence that they could cause kidney damage. More sensitive biomarkers also could make it possible for patients to be exposed to drugs that cause kidney damage in some individuals because exposure could be halted at the first sign of toxicity while the injury were still reversible.

Table 1. Predictive Safety Testing Consortium members.

Abbott Laboratories (NYSE:ABT)
Amgen Inc. (NASDAQ:AMGN)
AstraZeneca plc (LSE:AZN; NYSE:AZN)
Boehringer Ingelheim GmbH
Bristol-Myers Squibb Co. (NYSE:BMJ)
ClinXus
Eli Lilly and Co. (NYSE:LLY)
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)
Iconix Biosciences Inc., part of Entelos Inc. (LSE:ENTL)
Johnson & Johnson (NYSE:JNJ)
Merck & Co. Inc. (NYSE:MRK)
Novartis AG (NYSE:NVS; SWX:NOVN)
Pfizer Inc. (NYSE:PFE)
Roche (SWX:ROG)
sanofi-aventis Group (Euronext:SAN; NYSE:SNY)
Schering-Plough Corp. (NYSE:SGP)
Wyeth (NYSE:WYE)

Source: *Critical Path Institute*

Preclinical use of the biomarkers will clearly be helpful for making go or no-go decisions prior to Phase I testing, but nevertheless it is only an interim step toward qualifying biomarkers for clinical uses.

Clinical qualification, which will require studies demonstrating that biomarker results reliably predict clinical outcomes, could support the inclusion of biomarker-based tests on product labels and facilitate the use of biomarkers by physicians to make treatment decisions.

“It is important to make a distinction between using biomarkers for internal decision making in a company for progressing a compound versus being qualified for regulatory decision making,” said John Orloff, SVP and head of U.S. drug and medical regulatory affairs at Novartis.

The pathway

The Novartis-FDA CRADA was designed to answer an important question the FDA did not address in a March 2005 biomarker guidance document: how to reclassify a “probable” valid biomarker as a “known” valid biomarker.²

The document divided valid biomarkers into three categories: exploratory, known and probable. A biomarker is known (acceptable for regulatory purposes) if there is “widespread agreement in the medical or scientific community about the physiologic, toxicologic, pharmacologic, or clinical significance of the results,” according to the guidance.

The guidance stated that a valid biomarker may be “probable,” and therefore not accepted by regulators, for three main reasons: because “data elucidating its significance may have been generated within a single company and may not be available for public scientific scrutiny,” because data on its significance are not conclusive or because data on its performance have not been independently verified.

An exploratory biomarker is a candidate for reclassification as a known or probable valid biomarker based on additional data.

The pathway outlined through the FDA-Novartis CRADA, and refined through the PSTC’s work, starts with a request to the agency to qualify a biomarker for a specific use. In response, the FDA recruits a biomarker qualification review team and the biomarker sponsor submits data on the proposed biomarker as a Voluntary Data Submission (VXDS).

Next, the FDA review team and the biomarker sponsor jointly design studies that are required to qualify the biomarker, and the sponsor submits the study results to the review team, which then accepts or rejects the biomarker for specific uses.

First submission

PSTC’s nephrotoxicity biomarker application, which incorporated data from the FDA-Novartis CRADA as well as data from FDA laboratories, is the first joint submission from a consortium to the FDA and the **European Medicines Evaluation Agency** (EMA), and it is the first VXDS submission to the FDA, according to William Mattes, C-Path’s director of toxicology.

“This is a landmark effort in the sense that existing data for promising markers were evaluated together with regulators, a scientific plan to address information gaps was developed and agreed upon, the experiments

“It is important to make a distinction between using biomarkers for internal decision making in a company for progressing a compound versus being qualified for regulatory decision making.”

—John Orloff, Novartis AG

were conducted, and the data were analyzed and submitted to regulators with proposals for particular ‘fit for use’ claims for each marker,” said Kevin Carl, senior associate director of drug regulatory affairs at Novartis.

Scientists at the FDA and EMA serve as consultants to the PSTC but are not members. Nevertheless, their active involvement is essential for the project’s success, Frueh said.

“I don’t think industry would be committing millions of dollars to conduct the experiments and generate the datasets needed to qualify bio-

markers if there wasn’t the recognition that this is going to be a plausible approach from a regulatory view,” he told *SciBX*.

EMA participation is important, “because you don’t want to have biomarkers that are useful in one geographic region and not in another,” Frueh said. Japanese regulators have observed PSTC nephrotoxicity biomarker meetings and may review data from the consortium’s future submissions, he added.

The selection and cross-validation processes “are all transparent,” noted C-Path president and CEO Raymond Woosley. “Everything is available to any scientist who wants to be part of this. We’ve set up a grand rounds process at FDA where scientists come in and discuss this with other scientists who haven’t been involved in it.”

Qualifying the biomarkers

The PSTC working group identified 23 potential preclinical urinary nephrotoxicity biomarkers. Based on advice from the FDA and EMA about the kinds of data required to qualify the markers, the consortium designed protocols for evaluating their sensitivity and specificity.

Preliminary work included gene expression analysis of kidney and liver tissue, as well as experiments to determine doses of the nephrotoxicants that would produce lesions.

The PSTC adopted a common system for grading lesions on a scale from one to five. In addition, the consortium agreed on terminology for classifying lesion types, as well as a scheme for assigning lesions to specific regions of the kidney.

When the dosing and assessment criteria were agreed, the PSTC began a series of identical experiments in rats.

Experiments were conducted with four doses (control, low, mid and high) of eight nephrotoxicants as well as two hepatotoxicants as controls. Study designs included multiple dose groups and were conducted for different periods of time, up to a maximum of 14 days of dosing (see **Table 2, “Toxicity studies”**).

In addition to histopathology, the gold standard for preclinical nephrotoxicity, analyses included in-life data such as food consumption and body weight, clinical chemistry including creatinine and BUN, and multiplex ELISA measurements on urine.

Based upon a candidate marker’s ability to identify particular types of kidney pathology, and on advice from the FDA and EMA, the PSTC whittled the 23 potential biomarkers down to 7 that were included in the initial submission, said Mattes.

Novartis contributed some of the seven markers and PSTC member **Merck & Co. Inc.** contributed others. Some were proposed by both companies, according to Mattes.

Table 2. Toxicity studies. The Predictive Safety Testing Consortium (PSTC) tested the sensitivity and specificity of proposed preclinical nephrotoxicity biomarkers in rats that were exposed to eight nephrotoxicants. In addition, the PSTC conducted identical studies using two hepatotoxicants that do not cause kidney damage: α -naphthylisothiocyanate (ANIT) and methapyrilene, to assess the ability of the biomarkers to discriminate between kidney and liver toxicities.

Nephrotoxicant	Tubule	Glomerulus	Collecting duct	Mode of toxicity
Gentamycin	X	(X)		Lysosomal phospholipidosis
Puromycin	X ^(2nd)	X		Damage to podocytes
Vancomycin	X			Oxidative stress (free radicals)
Doxorubicin	X ^(2nd)	X		Oxidative stress to glomerulus filtration membrane
Furosemide	X			Mineralization
Lithium carbonate	X	(X)	X	Influences formation of intracellular cyclic adenosine monophosphate
Cisplatin	X	(X)	(X)	Direct DNA alkylation (oxidative stress)
Tacrolimus	X	(X)		Complex (vasoconstriction, calcification)

X signifies that the agent is directly toxic to the particular renal structure. (X) signifies that the agent may be directly toxic to that particular renal structure, but either to a lesser extent than a structure marked with X or only under certain conditions. X^(2nd) signifies that the agent is indirectly toxic to that particular renal structure (that is, its effects on primary renal structure result in toxicity to the secondary renal structure). Source: Novartis AG presentation to the Institute of Medicine

The consortium may submit other kidney toxicity biomarkers from the list to the FDA and EMEA in the future, according to Carl (see Table 3, “Kidney toxicity biomarkers”).

Preliminary PSTC data presented by Novartis at an **Institute of Medicine** meeting in April 2007 illustrated the sensitivity of some of the submitted biomarkers.

Using cisplatin, a chemotherapeutic that is known to cause serious kidney damage, Novartis reported that creatinine was elevated above the control threshold only when 3 mg/kg of drug, the highest dose, was administered; lower doses did not produce a toxicity signal based on creatinine.

Moreover, the creatinine test detected only grade three lesions, indicative of the worst kidney injury.

In contrast, Kim-1, a protein that is undetectable in healthy kidneys but is present in those that have suffered ischemic or toxic injury, was sensitive to a lower dose and to less-severe lesions. The Kim-1 marker was detected in rats exposed to 1 mg/kg of cisplatin, the middle dose, and histopathology examination determined that it was associated with grade one and two lesions. In addition to increased sensitivity, many of the submitted biomarkers signal damage to specific parts of the kidney. For example, urinary cystatin C predicts glomerular alteration, whereas clusterin signals distal and proximal tubule damage.

Regulatory decisions

The PSTC submitted its nephrotoxicity application to the FDA and EMEA on June 12, 2007. The FDA will notify the PSTC of its decisions “very soon,” according to Frueh.

An EMEA decision on the biomarker qualification applications is also “imminent,” according to Novartis.

The FDA is clearly impressed by both the results and the process used to obtain them. “The data are outstanding; there is no argument over how useful it is going to be,” Frueh told *SciBX*.

Following FDA and EMEA approval of the PSTC submission, the consortium’s next steps could include further qualification of other kidney biomarkers, probably involving clinical studies, and greater integration of clinical and preclinical data, Mattes said.

This summer, Novartis plans to submit additional proposed preclinical genomic and serum biomarkers of nephrotoxicity that are not part of the PSTC submission, Carl said. Novartis and the PSTC also plan to begin work to qualify some of the tests as valid clinical biomarkers.

“Because of the promise the new markers have shown in the data already generated, further translational studies are planned by Novartis and other members of the PSTC in order to

generate the needed data to eventually have the markers qualified for broad clinical use,” Carl told *SciBX*.

From the FDA’s point of view, qualification of preclinical markers is analogous to approving an IND, according to Frueh.

Approval of the PSTC application “will mean that now we have investigational biomarkers,” he said. “That is not the same as saying they should

be used broadly in clinical trials—we aren’t there yet, but clearly this is where we want to go.”

Qualification as clinical biomarkers could be based on studies with drugs like cisplatin that are nephrotoxic but are not fatal, according to Frueh. “You can imagine straightforward experiments where a biomarker was measured in people who take drugs in order to assess the level of toxicity,” he said.

Successful demonstration of the utility of the biomarkers in a clinical trial, for example using them to detect kidney damage early enough to stop exposure to a drug before it causes permanent kidney damage, could lead to approval of their inclusion on a drug label, Frueh said.

The markers “are assessments at the time damage occurs,” he noted, but the FDA might consider them “predictive in the sense that if there is continued exposure, you would expect these markers to go up further.”

“We hope the next step will demonstrate the usefulness of these markers in the clinical context. Then we will have true translational markers.”

—Felix Frueh, FDA

Table 3. Kidney toxicity biomarkers. The Critical Path Institute's Predictive Safety Testing Consortium (PSTC) has studied 23 urinary nephrotoxicity biomarkers. In July 2007, it submitted seven to the FDA and EMEA for qualification as preclinical biomarkers, shown in the first list below, and may submit others in the future. Histopathology studies demonstrated associations between many of the biomarkers and damage to specific areas of the kidney (glomerulus, proximal tubule, distal tubule, loop of henle and collecting duct).

Submitted biomarkers	Area of kidney	Description
Albumin	Not applicable	Protein found in blood plasma and urine that transports small molecules
β 2-microglobulin	Glomerulus; proximal tubule	Low molecular weight protein that is eliminated by glomerular filtration but completely reabsorbed by the tubules
Clusterin	Distal tubule; proximal tubule	Highly conserved protein associated with apoptosis
Cystatin C	Glomerulus	Nonglycosylated protein found in all nucleated cells; inhibitor of the elastin- and collagen-degrading cysteine proteases
Kim-1	Proximal tubule	Membrane protein implicated in damage and repair
Trefoil factor 3	Not applicable	Protein secreted onto mucosal surfaces; has tissue-specific activity including inhibiting apoptosis and modulating mitogenic activity
Total urinary protein	Not applicable	Amount of protein found in urine
Other biomarkers studied	Area of kidney	Description
Calbindin d28	Collecting duct; distal tubule	Vitamin D-dependent calcium binding protein
Epidermal growth factor	Distal tubule; proximal tubule	Growth factor that plays a role in cell system regeneration and acute renal injury recovery
Glutathione S-transferase- α	Proximal tubule	Cystolic detoxification enzyme
Glutathione S-transferase- μ	Distal tubule	Cystolic detoxification enzyme
Interferon- γ -induced 10 kDa protein	Not applicable	Chemokine that mediates the proliferation of human mesangial cells (HMC)
Lipocalin 2 (neutrophil gelatinase-associated lipocalin; NGAL)	Proximal tubule	Transporter protein that is upregulated during inflammation
Macrophage migration inhibitory factor	Not applicable	Tautomerase that is involved in development of septic shock, arthritis and glomerulonephritis
Monokine induced by interferon- γ	Not applicable	Chemokine chemoattractant for activated T cells
N-Acetyl- β -D-glucosaminidase	Proximal tubule	Lysosomal enzyme
Osteoactivin	Not applicable	Cell-surface and lysosomal protein involved in bone matrix production
Osteopontin	Distal tubule; loop of henle; proximal tubule	Acidic protein that binds to hydroxylapatite and is implicated in mediating the binding of osteoclasts to the mineral matrix of bone surfaces
Podocin	Glomerulus	Slit diaphragm protein that binds the cytoplasmic domain of nephrin
Replication protein A	Not applicable	Protein involved in DNA repair
Tissue inhibitor of metalloproteinase-1	Distal tubule; proximal tubule	Metalloproteinase inhibitor involved in the regulation of bone modeling
Uromodulin (Tamm-Horsfall)	Loop of henle	Glycoprotein with potential role in regulating cytokines
VEGF	Not applicable	Polypeptide that stimulates new blood vessel formulation

Source: Critical Path Institute and Novartis AG

Frueh said the ability to detect kidney injury while it is reversible could make it possible to salvage drugs that have been shelved because of fears they cause irreversible damage. The goal is "to be able to use this type of information to move compounds that are on hold, are stuck, or have been discontinued because of preclinical toxicity," he said.

"Preclinical qualification is the necessary first step," Frueh concluded. "We hope the next step will demonstrate the usefulness of these markers in the clinical context. Then we will have true translational markers."

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European Medicines Evaluation Agency, London, U.K.
Food and Drug Administration, Rockville, Md.
Iconix Biosciences Inc., Mountain View, Calif.
Institute of Medicine, Washington, D.C.
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Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
SRI International, Menlo Park, Calif.
University of Arizona, Tucson, Ariz.

Cancer: stay hungry

By Lev Osherovich, Senior Writer

A study in the *Proceedings of the National Academy of Sciences* points to a new strategy to protect normal cells from the toxic effects of chemotherapy. The report, from a team at the **University of Southern California**, shows that short-term starvation boosts chemotherapy resistance in normal cells but not in tumors.¹ Academic researchers are starting a clinical trial of the starvation therapy and hope to commercialize their discoveries through a new company they are forming.

The findings have piqued the interest of at least one cancer company, **Threshold Pharmaceuticals Inc.** Charles Hart, director of biology at Threshold, said the *PNAS* paper points to a conserved mechanism in eukaryotes for boosting drug tolerance through dietary restriction.

Chemotherapeutics poison growth-related processes, typically elevated in tumor cells, such as DNA replication and anabolic metabolism. However, toxicity to normal dividing cells is an unavoidable side effect. Thus, there is a need for adjuvants that improve the therapeutic index of chemotherapeutics, which is the relative toxicity to tumors vs. to normal cells.²

Lead author Valter Longo, associate professor of gerontology and Alzheimer's disease research at USC, told *SciBX* that the unexpected cancer finding arose from his lab's primary interest in how organisms fed a calorie-poor, near-starvation diet display longer survival and greater stress resistance than fully fed controls.³

The process of dietary restriction is thought to enhance longevity and stress resistance through signaling pathways that slow down growth in anticipation of lean times ahead. Longo's group and others previously identified some of these pathways in yeast.^{4,5} Among these are the Ras and Akt/S6K pathways, which are often deregulated in tumors.

The team began by testing whether dietary restriction could protect yeast cells from oxidative stress. The researchers used mutant yeast strains that mimic the effects of dietary restriction. When cultured in the presence of the generic chemotherapeutics methyl methanesulfonate or cyclophosphamide, the mutant cells proved more resistant to oxidative stress than the wild-type cells.

Longo then hypothesized that tumor cells do not pick up on starvation cues and would thus gain no benefit from dietary restriction.

"Cancer cells do not need growth factors to grow and they don't obey orders to stop," he said. Exploiting this recalcitrance to boost the therapeutic index of chemotherapy "was the first thing that came to mind," he added.

Longo's team examined how chemotherapeutics affected the survival of primary cultured glial cells compared with the survival of glioma cell lines under conditions of dietary restriction. Growth in low-glucose medium for 24 hours protected the primary cultured cells but not the tumor cells from high doses of cyclophosphamide.

Similar results were seen in mice injected with human neuroblastoma cells. Mice deprived of food for 48 hours tolerated higher doses of etoposide and survived significantly longer than fully fed control mice. Etoposide is a generic alkylating agent that inhibits topoisomerase II.

"Cancer cells do not need growth factors to grow and they don't obey orders to stop."

—Valter Longo,
University of Southern California

"This paper is excellent in terms of in the number of different systems tested, from yeast to primary cell culture to mice to human tumor xenografts," Threshold's Hart told *SciBX*. "But to me the *in vivo* animal studies were most dramatic."

Mice that had undergone short-term starvation "could tolerate lethal doses of etoposide," he noted.

Unknown mechanism

What isn't yet known is the mechanism by which starvation induces tolerance to chemotherapy.

Peter DiStefano, CSO of **Elixir Pharmaceuticals Inc.**, told *SciBX* that Longo's findings are hard to interpret without a specific molecular target in hand.

"Short-term starvation is probably triggering a program that improves stress resistance," he said. "I think it's really important, but mechanistically, I just don't know how it works."

DiStefano suggested that in addition to the Ras and Akt/S6K pathways, there might be other stress-related and diet-related mechanisms at play in Longo's experiment. These could include insulin signaling, adenosine monophosphate kinase, mammalian target of rapamycin and sirtuins.

Dietary restriction-induced chemotherapy resistance in healthy cells could result from modulation of any or all of these pathways, he said.

"We think there are lots of nodes in the network, and nobody has a single smoking gun," said DiStefano. "They're all important."

According to DiStefano, the next step would be to repeat the dietary restriction experiments in mouse models that have disruptions in each of these known pathways. Mutants that do not have improved chemotherapeutic resistance in response to starvation would point to the most relevant biological targets for pharmaceutical development.

Elixir is interested in dietary restriction as a possible approach to lowering blood sugar. Elixir's lead compound is mitiglinide, a potassium channel blocker that stimulates insulin secretion. Mitiglinide, which is marketed in Japan by **Kissei Pharmaceutical Co. Ltd.** as Glufast, is in Phase III testing for type 2 diabetes in the U.S.

Another company, **Sirtris Pharmaceuticals Inc.**, is also developing compounds that mimic dietary restriction—lowering blood glucose levels in hopes of treating type 2 diabetes and, potentially, aging. The lead compound is SRT501, a formulation of resveratrol that agonizes the sirtuin family histone deacetylase SIRT1. SRT501 is in Phase I testing for type 2 diabetes. Sirtris is being acquired by **GlaxoSmithKline plc.**

Neither Elixir nor Sirtris has tested its compounds as chemotherapy adjuvants. Elixir's DiStefano told *SciBX* that without a specific drug target, he doubts that this is something his company "can sink our teeth into."

Threshold's Hart also suggested it will be necessary to test the effect of short-term starvation on the pharmacodynamics and bioavailability of chemotherapeutics.

Hart also wants to know whether short-term starvation improves the therapeutic index of chemotherapeutics that work by different mechanisms

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Debating PD transplants

By Michael J. Haas, Senior Writer

Clinicians have been transplanting dopaminergic cells into patients with Parkinson's disease for about 20 years, but they haven't followed exactly what happens to those implants for any longer than four years after surgery.¹⁻⁴ Three papers in *Nature Medicine* now show the longer-term fate of those cells, based on results of postmortem histological analyses in people with Parkinson's disease who died of causes unrelated to the disease.⁵⁻⁷

Two of the papers show evidence of PD-associated aggregates in a small fraction of the transplanted cells, whereas the third does not.

.....
(Continued from "Cancer: stay hungry," p. 6)

than etoposide, such as "antimetabolites, growth factor receptor antagonists, microtubule-targeting agents and kinase inhibitors."

USC's Longo said short-term starvation does protect normal cells from chemotherapeutics that were not reported in the *PNAS* article. "I expect that it will work with the majority but not necessarily all chemo drugs," he said.

Hart said Threshold's 2DG, a nonmetabolizable glucose analog, has been shown to be a pharmacological mimic of dietary restriction. He said 2DG can trigger dietary restriction-like physiological effects,⁶ in addition to killing cancer cells.

The *PNAS* results are "really in our arena," Hart said. 2DG is in Phase I trials to treat solid tumors. Hart added that in Threshold's trial, 2DG is primarily acting as an antiglycolytic agent, not as a mimic of dietary restriction.

Starved for attention

Researchers from both industry and academia told *SciBX* that regardless of the actual mechanism by which short-term starvation improves the therapeutic index of chemotherapies, the approach published in *PNAS* has a clear route to the clinic. They noted that, instead of starving cancer patients, compounds that mimic the effects of dietary restriction could be used in combination with chemotherapy.

"I think this study raises the exciting possibility that short-term fasting could be useful in combination with chemotherapy in people," said Matt Kaerberlein, assistant professor of pathology at the **University of Washington**. "I'm cautiously optimistic about this approach. I'm not sure there are any significant barriers to bringing this to clinical trials."

Longo said the results warrant clinical follow-up. He is collaborating with clinicians to start a 20-patient trial to test the effect of short-term starvation on chemotherapeutic toxicity in blood cells and other tissues. The trial will be run at the **Norris Comprehensive Cancer Center** at USC and will exclude patients with cachexia, a wasting syndrome.

Cachexia aside, fasting poses a risk to already weak cancer patients, and Longo agreed a pharmacological mimic of dietary restriction is a

The issue at hand is how to explain those results and whether different types of transplants could avoid the problem of aggregate accumulation altogether.

Collectively the three studies examined transplants in 8 patients who passed away 9–16 years after receiving dopaminergic cell grafts transplanted from fetal midbrains.

Though its underlying cause is unknown, PD is characterized by protein aggregates that accumulate in, and eventually kill, dopaminergic neurons over the course of years or decades. Current therapies aim to counteract the resulting dopamine deficiency—for example, by using 3,4-dihydroxy-L-phenylalanine (L-dopa)—which can reverse symptoms and slow, but not halt, disease progression.⁸

Among the alternative treatments developed in the past two decades are cell-based therapies in which functional, dopamine-producing neurons are surgically implanted in the brains of people with PD, thereby compensating for the dysfunctional neurons. Such therapies
(Continues on p. 8)

more attractive option. Moreover, a therapeutic could produce greater levels of protection by homing in on the specific effectors of dietary restriction.

"As you move to the dietary restriction drug targets, you develop more specificity," he said.

Longo said his group has identified two promising drug formulations that "maintain a broad effect against multiple chemotherapeutics," thus mimicking the effects of the starvation treatment. The two drugs are marketed for undisclosed indications, but Longo said USC has patented the use of these formulations as well as short-term starvation as an adjuvant to cancer treatment. The patent also covers the modulation of the genetic pathway that mediates the effect of short-term starvation, according to Longo.

Longo has founded a company, **DSR Pharmaceuticals Inc.**, to commercialize the findings reported in *PNAS*. A forthcoming publication will describe the company's lead compounds and their mechanism of action, he said. Meanwhile, Longo is seeking investors for DSR.

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COMPANIES AND INSTITUTIONS MENTIONED

- DSR Pharmaceuticals Inc.**, Los Angeles, Calif.
- Elixir Pharmaceuticals Inc.**, Cambridge, Mass.
- GlaxoSmithKline plc** (LSE:GSK; NYSE:GSK), London, U.K.
- Kissei Pharmaceutical Co. Ltd.** (Tokyo:4547), Nagano, Japan
- Norris Comprehensive Cancer Center**, Los Angeles, Calif.
- Sirtris Pharmaceuticals Inc.** (NASDAQ:SIRT), Cambridge, Mass.
- Threshold Pharmaceuticals Inc.** (NASDAQ:THLD), Redwood City, Calif.
- University of Southern California**, Los Angeles, Calif.
- University of Washington**, Seattle, Wash.

deliver longer-term symptomatic relief than drugs like L-dopa.

But until now, it was not known how many of the transplanted cells continued to produce dopamine—or for how long.

The main molecular culprit in PD is α -synuclein, a protein that occurs naturally in neural tissue in a soluble form. In PD, α -synuclein becomes insoluble and precipitates inside neurons, where it associates with ubiquitin and other proteins to form aggregates called Lewy bodies. The proteasome cannot degrade Lewy bodies, so they accumulate in the neuron, where they impede the cell's normal functions and eventually kill it.

The presence of Lewy bodies in neurons, as indicated by histological analysis, is indicative of PD but not conclusive. That's because Lewy bodies occur in other, nonparkinsonian forms of dementia.

A European research team at **Lund University Hospital, Wallenberg Neuroscience Center** and **University College London** found Lewy bodies in 2–5% of the neurons transplanted into 2 patients, one who had survived for 11 years and the other for 16 years after surgery.⁵ The team was led by Patrik Brundin, professor of neurobiology and group leader of the Neuronal Survival Unit at Wallenberg.

A U.S. research team at **Mount Sinai School of Medicine, Rush University Medical Center** and the **University of South Florida** found Lewy body-like pathology in the transplanted neurons of a single patient who had survived for 14 years after surgery.⁶ The team was led by Jeffrey Kordower, professor of neurosurgery and director of the Research Center for Brain Repair at Rush.

Finally, a North American team studied 5 patients who had survived 9–14 years after transplant surgery and found no signs of pathology in the grafts.⁷ This team included researchers from **Dalhousie University, Queen Elizabeth II Health Science Centre, McGill University, McConnell Brain Imaging Centre, Montreal Neurological Institute, Harvard University** and **McLean Hospital**, and the **University of Pennsylvania**. The team was led by Ole Isacson, professor of neurology and director of Neuroregeneration Laboratories and the Center for Neuroregeneration Research at Harvard and McLean.

Habeas corpus

The Brundin and Kordower teams both saw aggregates resembling Lewy bodies in their patients' transplants, leading the teams to suggest that PD had spread from the host brain to the fetal midbrain grafts.

A key question is whether the pathology was caused by disease propagation from host to graft or by variations between the transplant methods. The answer could lead to improvements in cell-based therapies and perhaps illuminate the mechanisms behind PD.

The Kordower team wrote that “our results suggest that grafted cells can be affected by the disease process and thereby might limit the long-term clinical benefit of these treatment approaches,” including stem cell grafts.

“I'm very convinced there is a Parkinson's-like pathology in the dopamine cell transplants,” Brundin told *SciBX*.

Kordower agreed. “Our data and Brundin's are virtually identical. Both show that transplants undergo Parkinson's disease-like changes.”

Kordower also said the findings of both teams settle a long-running debate among PD researchers. The data, he said, show that the disease results from an ongoing process that continues to affect normal neurons throughout life and not from an acute insult to brain tissue that leads to long-term degeneration.

Marc Rubin, president and CEO of **Titan Pharmaceuticals Inc.**, agreed with Brundin and Kordower. “Basically we're seeing that the disease process—whatever its pathophysiology—is ongoing and affects fetal midbrain implants. It could conceivably affect implants derived from stem cells in the same way.”

“Basically we're seeing that the disease process—whatever its pathophysiology—is ongoing and affects fetal midbrain implants. It could conceivably affect implants derived from stem cells in the same way.”

**—Marc Rubin,
Titan Pharmaceuticals Inc.**

Titan is developing a cell-transplant therapy that uses human retinal pigmented epithelial cells (RPEs) as “dopamine microfactories in the brain.” Because RPEs are not neural cells, they don't make connections to other neurons—and so should not be susceptible to the PD process, he said.

The therapy is in a Phase IIb trial with partner Bayer Schering Pharma AG, a subsidiary of **Bayer AG**, with data expected this year.

Conversely, the Isacson team found no pathology in their patients' transplants and wrote that their results indicate that “the host brain does not necessarily create conditions

that cause Parkinson's disease-related neurodegeneration in the transplanted neurons.”

Curt Freed, professor and head of clinical pharmacology and toxicology and director of the Neurotransplantation Program for Parkinson's Disease at the **University of Colorado School of Medicine**, said his experience matched Isacson's results.

“I believe that we have done the largest series of dopamine cell transplants in the world—a total of 61 patients since 1988—and we have seen no protein deposits in dopamine neurons up to 14 years after transplant. Since the mechanism by which Parkinson's develops is unknown in most patients, and because the precipitation of α -synuclein and ubiquitin proteins is not unique to Parkinson's,” there is no reason to attribute the observed pathology to Parkinson's disease progression, he said.

Freed said the results of his study will be reported in a forthcoming publication.

Brundin acknowledged that there are other explanations besides PD for the pathology his team observed. “You could play devil's advocate and say that the pathology is not related to Parkinson's disease, that it could be related to aging,” he said. “We don't have controls in the usual sense, so we don't know whether we would see the same pathology in normal patients after 16 years. But I do see a potential causal link because in these cases we already know the patients have Parkinson's disease—so we have to consider disease propagation a strong possibility.”

Brundin offered an alternative explanation for the difference between studies.

“The number of cells that had survived in the 14-year patient [in Isacson's study] was about 9,800,” Brundin said. “Kordower and our group had access to 10 to 15 times as many cells, because the

transplant sizes were larger. In our studies, the proportion of dopaminergic transplant cells having Lewy bodies was between roughly 2% and 5%. When you apply this to 9,800 cells, I think Isacson's team may have just missed spotting the Lewy bodies."

Inflammatory reactions

In contrast, both Freed and Isacson attribute the pathology seen by the Brundin and Kordower teams to differences in transplant methods. Fetal midbrain cells can be transplanted as solid pieces, as cell suspensions or as tissue strands.

All the patients studied by Isacson's team received suspensions of midbrain cells. The patients in the Kordower and Brundin studies received solid pieces of midbrain.

Isacson said his team thinks solid-piece transplants induce an immune response, as indicated by earlier studies that found accumulation of microglia—the brain's immune cells—in such grafts.^{2,3} Those studies were conducted by Freed and by Kordower team member C. Warren Olanow, professor and chairman of neurology and professor of neuroscience at Mount Sinai.

The latest Kordower team wrote in *Nature Medicine* that they observed microglial activation in their patient's graft "to a degree that far exceeded the expression of microglia" in the surrounding host tissue. The Brundin team observed some microglia accumulation in their two patients' grafts, but wrote that the "immunohistochemistry did not indicate strong activation."

Isacson's team saw little indication of a microglial reaction to the transplanted cell suspensions. Thus, his group suggested that extracellular matrix and other non-neural tissue in solid pieces induce a chronic host inflammatory response that results in degeneration of the implanted cells over time.

"Individual differences in chronic inflammatory reactions around the implanted cells that are patient- or method-specific variations have been found, which could account for differences in protein aggregates" noted among the three papers, Isacson said. Current and future studies should determine how best to minimize inflammatory response, he added.

Michel Levesque, president and CEO of **NeuroGeneration Inc.**, agreed with Isacson. "The presence of microglia indicates an immune reaction to the grafts that caused cell dysfunction and initiated the formation of Lewy bodies," he said.

He also said the grafts develop pathology in the same way as the original host cells do—as a result of "life-long exposure of the cells to oxidative stressors," which could include chemicals known to cause PD-like pathology.

"I think that the immune reaction and exposure to oxidative stressors are two different mechanisms that both induce upregulation of α -synuclein, and their effect is additive," Levesque said.

NeuroGeneration is developing a transplant therapy for PD that uses neurons derived from the patient's own neural stem cells. According to Levesque, the implanted cells are a mixture of dopaminergic neurons and neurons that produce other neurotransmitters, such as γ -aminobutyric acid (GABA). Using a patient's own cells should avoid immunogenicity issues, he said.

Levesque said the company's Phase II trial of its neural stem cell therapy is on hold awaiting the completion of a GMP-compliant cell processing facility and should resume within six months.

Erik Miljan, head of stem cell discovery at **ReNeuron plc**, said the quality of the cell material used also could affect whether transplanted cells develop pathology.

In the three papers, "it is generally assumed that primary tissue isolated directly from fetal donor is the highest potency because it is from a developing brain and not manipulated," he said. "However, uncontrolled variables arise—for example, when multiple donors are used for one patient and the tissues must be stored until enough is collected."

Ideally, the potency and efficacy of the cells for implantation should be assessed by *in vitro* assays and *in vivo* studies in animal models.

"Unfortunately, the limited amount of material that is available from a fetal donor frequently precludes such testing because all of the material is required for the treatment," he said.

Freed said his group has incorporated this kind of assessment into their transplantation method. "We collect tissue up to one month before transplant, holding it in tissue culture so that we can make sure the tissue is not infected

and that it is making dopamine," he said. "No other group does this testing. The method also gives us time to accumulate multiple samples and schedule surgery as an elective—rather than emergency—surgery."

ReNeuron has human stem cell lines (ReN004) in discovery research for PD. Miljan anticipates that such cell lines would provide a continuous, renewable and standardized source of dopaminergic cells.

Troubles in paradigm?

One question at least one team plans to tackle is how the Lewy bodies show up in the transplanted cells.

"Now we have to understand why and how Lewy bodies form in grafts," Brundin said. "Knowing the mechanism could help us to design stem cell-derived grafts that are less prone to Lewy body formation."

He added: "Perhaps an understanding of the molecular basis for the host-to-graft propagation of pathology could also help us devise therapies to slow progression of the disease in patients without transplants."

Brundin said his team is planning *in vitro* and *in vivo* studies to investigate the mechanism by which PD pathology propagates from host to graft cells.

NeuroGeneration's Levesque noted that "not much is known about the brain's immune response to implants. The findings [in the three papers] suggest that current cell-based therapies may not be the best methods" because any cell source foreign to the host will be immunogenic. This is why NeuroGeneration is developing autologous stem cell therapies, he said.

Levesque also thinks the medical community and industry operate from a PD paradigm that is too narrow and could therefore limit researchers' ability to explain the differences between the three *Nature Medicine* papers. The PD community has "simplified Parkinson's disease to the extreme by describing it as a dopamine deficiency," he said.

"We have to approach the disease differently, with an understanding of all of the modified neurotransmitters in Parkinson's disease—not

"Now we have to understand why and how Lewy bodies form in grafts."

**—Patrick Brundin,
Wallenberg Neuroscience Center**

just dopamine—and how these modifications change the brain,” he said.

For instance, he noted L-dopa is thought to create an imbalance between dopamine and GABA in the dopamine-deficient brain. “The chronic imbalance leads to dyskinesia and motor function complications resulting from the drug itself,” he said. He added that chronic intake of drugs like L-dopa is also suspected to cause damage to neurons.

Cell-based therapies, like NeuroGeneration’s, that produce a mixture of neurotransmitters should do a better job of treating the whole disease process than do therapies that focus only on restoring dopamine production, Levesque said.

Nevertheless, Kordower noted “the vast majority of transplanted cells survive. So yes, this means that stem cell research should go forward.”

Kordower sits on the scientific advisory board of **Ceregene Inc.**, which has CERE-120, an adeno-associated virus type 2 vector encoding the *neurturin* (*NTN*) gene, in Phase II testing for PD with partner **Genzyme Corp.**

“Overall evidence points to robust, long-term survival of implants in all studies,” Isacson said. He said his *Nature Medicine* coauthor Ivar Mendez—who is head of neurosurgery at both Dalhousie University and Queen Elizabeth II Health Sciences Centre—is leading a team to develop new methods of preparing fetal cells, surgical improvements and stem cell-derived dopaminergic neurons.

“I find it remarkable that all three *Nature Medicine* reports, and our experience in Colorado, show that dopaminergic cell transplants survive and function indefinitely despite the differences in methods,” Freed said. “This is a positive, not a cautionary, story.”

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e-mail: isacson@hms.harvard.edu
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Bayer AG (Xetra:BAY), Leverkusen, Germany
Ceregene Inc., San Diego, Calif.
Dalhousie University, Halifax, Nova Scotia, Canada
Genzyme Corp. (NASDAQ:GENZ), Cambridge, Mass.
Harvard University, Belmont, Mass.
Lund University Hospital, Lund, Sweden
McConnell Brain Imaging Centre, Montreal, Quebec, Canada
McGill University, Montreal, Quebec, Canada
McLean Hospital, Belmont, Mass.
Montreal Neurological Institute, Montreal, Quebec, Canada
Mount Sinai School of Medicine, New York, N.Y.
Queen Elizabeth II Health Science Centre, Halifax, Nova Scotia, Canada
NeuroGeneration Inc., Los Angeles, Calif.
ReNeuron plc (LSE:RENE), Guildford, U.K.
Rush University Medical Center, Chicago, Ill.
Titan Pharmaceuticals Inc. (AMEX:TTP), South San Francisco, Calif.
University College London, London, U.K.
University of Colorado School of Medicine, Denver, Colo.
University of Pennsylvania, Philadelphia, Pa.
University of South Florida, Tampa, Fla.
Wallenberg Neuroscience Center, Lund, Sweden



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This week in therapeutics

THE DISTILLERY brings you this week's most essential scientific findings in therapeutics, distilled by *SciBX* editors from a weekly review of more than 400 papers in 38 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				
Cancer	IκB kinase (IKK); NF-κB; type 1 glucose transporter (GLUT3)	A study in cell culture suggests that blocking or downregulating IKK activity could help treat some cancers. In p53-deficient mouse embryonic fibroblasts, enhanced IKK and subsequent NF-κB activity were associated with a higher rate of aerobic glycolysis and upregulation of GLUT3, a glucose transporter protein, compared with those seen in wild-type fibroblasts. Also, a glycolytic inhibitor suppressed IKK activity in p53-deficient cells, suggesting a positive-feedback loop. Higher rates of aerobic glycolysis often help sustain tumor growth in human cancers lacking p53. Next steps include analyzing the mechanism by which glycolysis drives IKK and NF-κB activation in the absence of p53. MLN0415, a small molecule IKK inhibitor from Millennium Pharmaceuticals Inc., is in Phase I testing to treat inflammatory diseases.	Not patented; unlicensed	Kawauchi, K. <i>et al. Nat. Cell Biol.</i> ; published online March 31, 2008; doi:10.1038/ncb1724 Contact: Nobuyuki Tanaka, Nippon Medical School, Kawasaki-shi, Japan e-mail: nobuta@nms.ac.jp
Cancer	p53 tumor suppressor pathway	A study in cell culture suggests that reactivation of the p53 tumor suppressor pathway could be a useful strategy for treating some cancers. In A431 human carcinoma lines that express mutant p53, treatment with the small molecule compound called reactivation of transcriptional reporter activity (RETRA) resulted in a smaller number of cancer cell colonies than were seen in similar cells that received dimethyl sulfoxide (DMSO) as control. In murine A431 xenografts, peritoneal injection of RETRA resulted in later onset of initial tumor formation and a lower total number of tumors compared with injection of DMSO control. Next steps include optimizing compounds similar to RETRA.	Not patented; unlicensed	Kravchenko, J.E. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online April 14, 2008; doi:10.1073/pnas.0802091105 Contact: P.M. Chumakov, The Cleveland Clinic Foundation, Cleveland, Ohio e-mail: chumakp@ccf.org
Cancer	Toll-like receptor 5 (TLR5)	A study in mice and rhesus monkeys suggests that TLR5 agonists might protect healthy cells from radiation and thus be useful as adjuvants to cancer radiotherapy or as a defense against ambient radiation. In healthy rhesus monkeys, a single injection of the TLR5 agonist CBLB502 significantly increased survival following total body gamma radiation compared with survival of monkeys receiving buffer control ($p<0.03$). In transgenic mouse models of cancer, CBLB502 plus irradiation did not negatively affect the sensitivity of tumors to radiotherapy. The next step is to move the agonists into human trials. Cleveland BioLabs Inc. has submitted an IND for CBLB502 and plans to begin a Phase I trial for biodefense and a Phase I/II trial in cancer patients receiving radiotherapy this year.	Multiple patent applications filed covering radioprotection using NF-κB activators of bacterial origin and methods to discover the apoptosis inhibitors; exclusively licensed to Cleveland BioLabs	Burdelya, L. <i>et al. Science</i> ; published online April 11, 2008; doi:10.1126/science.1154986 Contact: Andrei V. Gudkov, Roswell Park Cancer Institute, Buffalo, N.Y. e-mail: andrei.gudkov@roswellpark.org Contact: Elena Feinstein, Cleveland BioLabs Inc., Buffalo, N.Y. e-mail: efeinstein@cblbiolabs.com

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer; thrombosis; osteoporosis	Rab geranylgeranyl transferase (RabGGTase)	<i>In vitro</i> studies identified inhibitors of RabGGTase that could be useful for treating thrombotic disorders, osteoporosis and cancer. A Rab prenylation assay of 469 tripeptide derivatives identified 4 highly selective RabGGTase inhibitors. Researchers also solved the first X-ray crystal structure of a RabGGTase-inhibitor complex. Next steps include using the structure in SAR studies to optimize the peptidomimetic inhibitors and determining whether any nonpeptide inhibitors show similar potency.	Inhibitors not patented; available for licensing	Guo, Z. <i>et al. Angew. Chem. Int. Ed.</i> ; published online April 11, 2008; doi:10.1002/anie.200705795 Contact: Herbert Waldmann, Max Planck Institute of Molecular Physiology, Dortmund, Germany e-mail: herbert.waldmann@mpi-dortmund.mpg.de Contact: Kirill Alexandrov, same affiliation as above e-mail: kirill.alexandrov@mpi-dortmund.mpg.de
Skin cancer	β -catenin	Studies in mice with tumors and mice with human xenografts suggest that antagonizing β -catenin could be useful for treating some forms of skin cancer. The Wnt/ β -catenin signaling pathway has been implicated in a number of human tumors. In mice with established skin tumors, β -catenin knockout resulted in complete tumor regression. In human squamous cell carcinoma (SCC) xenografts, small hairpin RNA knockdown of β -catenin significantly reduced tumor growth compared with the use of control shRNA ($p < 0.0008$). Next steps include identifying the signaling targets of β -catenin required for tumor promotion and cancer stem cell maintenance, as well as screening various libraries for potential inhibitors of the signaling pathway.	Not patented; available for licensing through the Swiss Federal Institute of Technology Lausanne Technology Transfer Department	Malanchi, I. <i>et al. Nature</i> ; published April 3, 2008; doi:10.1038/nature06835 Contact: Joerg Huelsken, Swiss Institute for Experimental Cancer Research and National Center of Competence in Research, Epalinges, Switzerland e-mail: joerg.huelsken@epfl.ch

Cardiovascular disease

Ischemia/ reperfusion injury; myocardial infarction (MI)	Receptor- associated adaptor kinase (RIP1)	An SAR study identified necrostatins as RIP1 inhibitors that could be useful for treating ischemia, MI and other pathologies associated with nonapoptotic cell death. Necrostatin-1, necrostatin-3 and necrostatin-5 all blocked nonapoptotic cell death <i>in vitro</i> through inhibition of the serine/threonine kinase activity of RIP1. Further studies are necessary to optimize the necrostatin compounds.	Multiple patent applications filed by Harvard University and Brigham and Women's Hospital covering therapeutic and diagnostic applications worldwide; available for licensing	Degterev, A. <i>et al. Nat. Chem. Biol.</i> ; published online April 13, 2008; doi:10.1038/nchembio.83 Contact: Junying Yuan, Harvard Medical School, Boston, Mass. e-mail: jyuan@hms.harvard.edu Contact: Alexei Degterev, Tufts University, Boston, Mass. e-mail: alexei.degterev@tufts.edu
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This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Hematology				
Sickle cell disease (SCD)	Endothelin receptor	<p>A study in mice suggests that endothelin receptor antagonists could be useful for treating SCD. SCD mice exposed to severe hypoxia and treated with the dual endothelin receptor antagonist bosentan had significantly increased survival compared with untreated mice (100% vs. 27%, $p < 0.001$). Bosentan also prevented renal and pulmonary microvascular congestion and systemic inflammation. Next steps include investigating the effects of specific endothelin receptor subtypes on abnormal vascular phenotypes in preclinical SCD models as well as determining the pathway for endothelin receptor activation and the effects of endothelin receptor antagonism on prevention of chronic organ damage.</p> <p>Tracleer bosentan from Actelion Ltd. is marketed to treat pulmonary arterial hypertension (PAH). Actelion provided bosentan for the study.</p> <p>Gilead Sciences Inc. markets selective endothelin A receptor antagonist Letairis ambrisentan in the U.S. to treat PAH. GlaxoSmithKline plc has commercialization rights outside of the U.S.</p> <p>Thelin sitaxsentan, an endothelin A receptor antagonist that Pfizer Inc. acquired from Encysive Pharmaceuticals Inc. earlier this year, is approved in Australia, Canada and Europe to treat PAH but has received three FDA approvable letters requesting additional clinical work for the indication in the U.S.</p>	Patent and licensing status undisclosed	<p>Sabaa, N. <i>et al. J. Clin. Invest.</i>; published online March 27, 2008; doi:10.1172/JCI33330</p> <p>Contact: Pierre-Louis Tharaux, Cardiovascular Research Center, Paris, France e-mail: tharaux@chusa.jussieu.fr</p>
Infectious disease				
HPV	HPV16 minor capsid protein L2	<p>A vaccine that contains an L2-derived peptide together with two immunostimulatory adjuvants could be useful for immunizing against HPV infection. The vaccine's active ingredient is a lipopeptide consisting of residues 17–36 of HPV16 minor capsid protein L2 linked to T helper cell epitope P25 and toll-like receptor 2 ligand dipalmitoyl-S-glyceryl cysteine (Pam₂Cys). In wild-type mice, subcutaneous and intranasal administration of the P25-Pam₂Cys-HPV lipopeptide elicited antibody responses against HPV16, HPV5, HPV18, HPV45 and bovine papillomavirus type 1. Future studies are needed to determine whether P25 is broadly recognized by diverse MHCs in the human population. Cervarix from GlaxoSmithKline plc and Gardasil from Merck & Co. Inc. are recombinant L1 virus-like particles (VLPs) that are marketed to prevent multiple HPV strains.</p>	Provisional patent on use of L2 for vaccine development; patent on L2 epitope filed; several companies have nonexclusively licensed the L2 epitope; currently negotiating exclusive licensing of L2 lipopeptide with VacTx	<p>Alphs, H.H. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online April 15, 2008 doi:10.1073/pnas.0800868105</p> <p>Contact: Richard B.S. Roden, Johns Hopkins School of Medicine, Baltimore, Md. e-mail: roden@jhmi.edu</p>
Malaria	Unknown	<p>An SAR study identified a series of 4(1H)-pyridone clopidol derivatives that could be useful for treating malaria. A number of the compounds had better antimalarial activity than the clopidol parent compound, against both murine <i>Plasmodium yoelii</i> in mice and human <i>P. falciparum</i> <i>in vitro</i>. Next steps include identifying pyridone compounds with increased antimalarial activity and studying the compounds in the clinic.</p> <p>GlaxoSmithKline plc's GSK93212, a 4(1H)-pyridone, is in preclinical development to treat malaria. At least 14 companies have compounds to treat malaria in preclinical and clinical development.</p>	Patented; not available for licensing	<p>Yeates, C. <i>et al. J. Med. Chem.</i>; published April 9, 2008; doi:10.1021/jm0705760</p> <p>Contact: Clive L. Yeates, Wellcome Research Laboratories, Kent, U.K. e-mail: cly@inpharma.co.uk</p> <p>Contact: José M. Bueno, GlaxoSmithKline R&D, Tres Cantos, Spain e-mail: jose.m.bueno@gsk.com</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Smallpox	Type I interferon binding protein (type I IFN bp)	A study in mice suggests that targeting type I IFN bp could be part of a strategy to vaccinate against smallpox infection. Type I IFN bp is secreted by poxviruses and is considered an immune response modifier that potentially contributes to virulence. In healthy wild-type mice, inoculation with recombinant type I IFN bp completely protected against mousepox infection, whereas mice inoculated with control antigen <i>Her2/neu</i> all died by day 10. Future studies are needed to determine whether the findings can be generalized to include other immune response modifiers and other members of the <i>Orthopoxvirus</i> genus. Multiferon, a type I IFN receptor agonist from Viragen Inc. and partner AFG BioSolutions Inc., is in preclinical testing as a smallpox vaccine.	Patent application filed; available for licensing	Xu, R.-H. <i>et al. J. Exp. Med.</i> ; published online April 7, 2008; doi:10.1084/jem.20071854 Contact: Luis J. Sigal, Fox Chase Cancer Center, Philadelphia, Pa. e-mail: Luis.Sigal@fcc.edu

Inflammation

Asthma	<i>Chitinase 3-like 1</i> gene (<i>CHI3L1</i>) on chromosome 1q32.1	A genome-wide association study identified a SNP in the promoter region of <i>CHI3L1</i> that could help diagnose asthma and other airway disorders. <i>CHI3L1</i> encodes chitinase-like protein YKL-40. In a founder population of 632 Hutterites, 4 different SNPs were all associated with high serum YKL-40 levels ($p=1.3 \times 10^{-12}$ to 1.1×10^{-13}), asthma ($p=0.047$ to 0.008) and bronchial hyper-responsiveness ($p=0.002$ to 5.9×10^{-4}). Three of these SNPs were nonrandomly linked with a fourth SNP located in the promoter region of <i>CHI3L1</i> . Next steps include exploring the potential of blocking YKL-40 as a therapy to treat asthma. <i>CHI3L1</i> genotyping might also be used to screen patients who would benefit from such therapy.	Not patented; unlicensed	Ober, C. <i>et al. N. Engl. J. Med.</i> ; published online April 9, 2008; doi:10.1056/NEJMoa0708801 Contact: Carole Ober, University of Chicago, Chicago, Ill. e-mail: c-ober@genetics.uchicago.edu
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Neurology

Amyotrophic lateral sclerosis (ALS)	Superoxide dismutase (SOD1) mRNA	A study in mice suggests that direct CNS delivery of short interfering RNA could be a useful strategy for treating ALS. In murine ALS models, spinal delivery of a chemically modified siRNA targeting SOD1 mRNA for four weeks from disease onset delayed early-stage and end-stage disease to 23 and 33 days, respectively, compared with 14 and 20 days for mice receiving buffer control. Direct CNS delivery caused minimal cytotoxic side effects. Next steps include testing the modified siRNA therapeutics in humans. Isis Pharmaceuticals Inc. and RXi Pharmaceuticals Corp. have RNAi therapeutics targeting SOD1 in preclinical development to treat ALS.	Research patented by University of Massachusetts Medical School; licensed to RXi Pharmaceuticals	Wang, H. <i>et al. J. Biol. Chem.</i> ; published online March 26, 2008; doi:10.1074/jbc.M800834200 Contact: Zuoshang Xu, University of Massachusetts Medical School, Worcester, Mass. e-mail: zuoshang.xu@umassmed.edu
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This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Pulmonary disease				
Inflammation	Nalp3 inflammasome; IL-1 β ; reactive oxygen species (ROS)	A study in mice and macrophage cell lines suggests that inhibition of the Nalp3 inflammasome could be useful for treating pulmonary inflammatory diseases linked to asbestos and other pathogenic air pollutants. In asbestos-exposed macrophage cell lines, ROS were produced and activated the Nalp3 inflammasome, leading to IL-1 β secretion. Both ROS and IL-1 β have been previously implicated in asbestos pathogenicity. In mouse models of asbestos inhalation, Nalp3 knockout resulted in less recruitment of inflammatory cells to the lung and less cytokine production than in wild-type mice. Next steps include investigating how the inflammasome senses extracellular asbestos and identifying other proinflammatory airborne pollutants that activate the Nalp3 inflammasome. Abbott Laboratories' DVD-Ig, an inhibitor of IL-1 α and IL-1 β , is in preclinical testing to treat rheumatoid arthritis (RA). AV411, a glial attenuator that suppresses IL-1 β from Avigen Inc., is in Phase II trials for pain.	Not patented; unavailable for licensing	Dostert, C. <i>et al. Science</i> ; published April 4, 2008; doi:10.1126/science.1156995 Contact: Jürg Tschopp, Department of Biochemistry, University of Lausanne, Epalinges, Switzerland e-mail: jurg.tschopp@unil.ch
Various				
Rhinitis; asthma; inflammatory airway conditions	Transient receptor potential cation channel, subfamily A, member 1 (TRPA1)	A study in cell culture and mice suggests that antagonizing TRPA1 could be useful for treating inflammation and pain associated with some airway diseases. In primary sensory neurons and heterologous cells, reactive oxygen species activated TRPA1 channels and induced Ca ²⁺ influx, showing that TRPA1 serves as a major neuronal sensor for oxidants in the airways. In TRPA1-null mice, reactive oxidants such as hydrogen peroxide had a lower capacity to induce hypoventilation and pain than they did in wild-type mice. Next steps include testing known TRPA1 antagonists in animal models of cough and in irritant exposure situations.	Patent application filed; available for licensing	Bessac, B. <i>et al. J. Clin. Invest.</i> ; published online April 8, 2008; doi:10.1172/JCI34192 Contact: Sven-Eric Jordt, Yale University School of Medicine, New Haven, Conn. e-mail: sven.jordt@yale.edu

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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 38 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
Chemotherapy with reduced side effects	<p>A study in yeast, multiple mammalian cell lines and mice suggests that combining standard chemotherapy with short-term starvation could reduce the toxic side effects associated with many cancer treatments. Healthy rat primary glia incubated in low-glucose media had significantly higher rates of survival than did healthy glia incubated in normal-glucose media following treatment with the chemotherapeutic cyclophosphamide (80% survival vs. 20%, $p < 0.01$). Multiple rat and human glioma lines had equally reduced rates of survival in both low- and normal-glucose environments after receiving cyclophosphamide compared with those seen in untreated lines. In mouse models of neuroblastoma, a 48-hour period of starvation followed by treatment with etoposide led to significantly higher survival rates compared with survival of untreated control mice on a normal diet ($p < 0.001$). Next steps include identifying the molecular mechanism of short-term starvation and developing a clinical protocol for short-term starvation in chemotherapy.</p> <p>Cyclophosphamide is a generic DNA-alkylating agent. Etoposide is a generic topoisomerase II inhibitor. Both drugs are marketed for multiple cancer indications. (See Cancer: stay hungry, page 6.)</p>	Patented; DSR Pharmaceuticals Inc. is negotiating to in-license the patents from the University of Southern California	Raffaghello L.R. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 31, 2008; doi:10.1073/plans.0708100105 Contact: Valter D. Longo, University of Southern California, Los Angeles, Calif. e-mail: vlongo@usc.edu
Flow cytometry-based short interfering RNA screen	<p>A flow cytometry-based siRNA screen could be useful for identifying kinases involved in cellular signal transduction pathways. The screen, which included siRNA constructs corresponding to all known human kinases, identified multiple kinases required for the interferon-γ response in human osteosarcoma U2OS cells. Scrambled siRNA and siRNA not processable by RNA-induced silencing complex helped control for off-target effects. Researchers did not disclose next steps.</p>	Patent and licensing status undisclosed	Watling, D. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online April 7, 2008; doi:10.1073/pnas.0710814105 Contact: A.P. Costa-Pereira, Imperial College London, London, U.K. e-mail: a.costa-pereira@imperial.ac.uk
Microscopy for cell adhesion dynamics	<p>Photoactivated localization microscopy (PALM) imaging could be useful for detecting transmembrane cytoskeleton-substrate attachment points that take part in cell migration, a process implicated in cancer and other diseases. The imaging method detected nanoscale dynamics within individual adhesion complexes in living cells for up to 25 minutes. The technique also measured the fractional gain and loss of the adhesion complex protein paxillin as each adhesion complex evolved. Researchers did not disclose next steps.</p>	Patent status undisclosed; Eric Betzig and Harald Hess, co-inventors of PALM, have licensed the rights to build and sell PALM microscopes to Carl Zeiss Inc.	Shroff, H. <i>et al. Nat. Meth.</i> ; published online March 16, 2008; doi:10.1038/nmeth.1202 Contact: Hari Shroff, Howard Hughes Medical Institute, Ashburn, Va. e-mail: shroffh@janelia.hhmi.org
PCR for detecting low-prevalence mutations	<p>Coamplification at lower denaturation temperature PCR (COLD-PCR) may offer improved sensitivity over traditional PCR for identifying low-prevalence mutations that occur in some cancers and infectious diseases, as well as identifying fetal alleles in maternal blood. The technique relies on variations in melting temperature caused by single-nucleotide mismatch to enrich for mutations against a wild-type background. Consequently, the downstream sensitivity of both Sanger sequencing and pyrosequencing were higher than that of standard PCR, and previously undetected mutations in the genes encoding p53, K-Ras and epidermal growth factor were identified in cancer samples. Further studies are necessary to adapt the method to specific genes of interest and package it into an assay for commercialization.</p>	Patent application filed covering COLD-PCR; available for licensing worldwide	Li, J. <i>et al. Nat. Med.</i> ; published online April 13, 2008; doi:10.1038/nm1708 Contact: G. Mike Makrigiorgos, Harvard Medical School, Boston, Mass. e-mail: mmakrigiorgos@partners.org

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Real-time tumor imaging	Real-time quantum dot (qdot) microscopy could provide high-resolution imagery of solid tumors. Fluorescent nanoparticles conjugated to an integrin-targeting peptide were injected into the tail vein of mice and bound tumor luminal epithelium as qdot aggregates. Next steps include investigating the phenomenon of qdot aggregates binding to tumor neovasculature, applying these methods to other tumor and nanoparticle types, and mathematical modeling to understand qdot binding to targets and migrating from vascular to extravascular spaces.	Not applicable	Smith, B. <i>et al. Nano Lett.</i> ; published online on April 4, 2008; doi:10.1021/nl080141f Contact: Sanjiv Sam Gambhir, Molecular Imaging Program at Stanford University, Stanford, Calif. e-mail: sgambhir@stanford.edu
Smart micelle carriers for delivering gene therapy	An <i>in vitro</i> study shows that nanoscale carriers consisting of plasmid DNA, cationic polymers and polyethylene glycol (PEG) could be useful for delivering gene therapy to diseased tissue. The PEG molecules of the smart polyplex micelles were engineered with disulfide linkages to ensure escape of plasmid cargo from the endosome following PEG detachment as a result of disulfide reduction. In HeLa and 293T cells, the micelles had 10-fold to 1,000-fold higher transfection efficiency and more rapid onset of gene expression than similar micelles without disulfide linkages. Further studies are necessary to test the potential of these vectors as systemically delivered cancer therapies and locally delivered tissue-regeneration therapies.	Patent application filed; available for licensing	Takae, S. <i>et al. J. Am. Chem. Soc.</i> ; published online April 9, 2008; doi:10.1021/ja800336v Contact: Kazunori Kataoka, The University of Tokyo, Tokyo, Japan e-mail: Kataoka@bmw.t.u-tokyo.ac.jp
Transdifferentiation of fibroblasts to create cells for tissue regeneration	A study in cell culture suggests that it may be possible to directly convert fibroblasts into other cell types and thus obviate the need for embryonic stem cells. Retroviral expression of two transcription factors, PU.1 and C/EBP α or C/EBP β , in mouse embryonic or primary fibroblasts was sufficient to convert the fibroblasts into cells with macrophage-like functional phenotypes, including phagocytosis of bacteria and a partial inflammatory response. Next steps could include using the approach to directly convert human skin biopsy cultures into cells of therapeutic interest.	Research not patented; unavailable for licensing	Feng, R. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online April 14, 2008; doi:10.1073/pnas.0711961105 Contact: Thomas Graf, Center for Genomic Regulation, Barcelona, Spain e-mail: thomas.graf@crg.es
Transgenic mouse model of human vasculature	Subcutaneous implantation of spheres of human endothelial cells into immunodeficient mice could be useful for modeling human vasculature and identifying antiangiogenic therapies. Human umbilical vein endothelial cell (HUVEC) spheres were embedded in a Matrigel-fibrin matrix containing growth factors VEGF and fibroblast growth factor-2 (FGF-2), which was subcutaneously implanted into mice. At 20 days post-transplant, the resulting implant vasculature consisted almost entirely of human endothelial cells, and functional microvessels had formed. At 60 days, the vasculature remained stable and responded to exogenous cytokines. In mice implanted with HUVECs, the VEGF receptor antagonist PTK787/ZK222584 significantly inhibited VEGF- and FGF-2-induced angiogenesis compared with that in untreated controls ($p < 0.05$). Further research is investigating the process of angiogenesis and the interactions of various tumor tissues and human endothelial cells. PTK787/ZK222584, an angiogenesis inhibitor from Novartis AG, is in Phase II testing to treat stage IIIB/IV non-small cell lung cancer (NSCLC).	ProQinase GmbH offers the procedure for compound testing purposes on a fee-for-service basis	Alajati, A. <i>et al. Nat. Meth.</i> ; published online on April 6, 2008; doi:10.1038/nmeth.1198 Contact: Hellmut Augustin, Department of Vascular Biology and Angiogenesis Research, Tumor Biology Center, Heidelberg, Germany e-mail: augustin@angiogenese.de
Tumor-targeting peptides	A study in mice suggests that using peptides as delivery vehicles could be useful for targeting inaccessible solid tumors and thus treating some cancers. In a transgenic mouse model of pancreatic cancer, i.v. peptide-mediated delivery of anti-CD40 antibodies and IL-2 to islet tumors led to accumulation of the compounds near tumor vasculature without toxic side effects. This resulted in reduced tumor vasculature and delayed tumor growth by enhancement of inflammatory and immune responses compared with using non-peptide mediated anti-CD40 antibodies and IL-2. Further studies are necessary to identify a tumor-homing molecule specific for human tumors, as the peptide used in the study is specific to mice.	Not applicable	Hamzah, J. <i>et al. J. Clin. Invest.</i> ; published online April 8, 2008; doi:10.1172/JCI33201 Contact: Ruth Ganss, Western Australian Institute for Medical Research, Perth, Western Australia, Australia e-mail: ganss@waimr.uwa.edu.au

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