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Moving Velcade into lupus

By Tim Fulmer, Senior Writer

Memory B cells and short- and long-lived plasma cells secrete autoantibodies that are thought to be at the root of lupus.[1,2] Short-lived plasma cells can be targeted indirectly by blocking B cell activation, but that approach has been disappointing, as illustrated by the recent failure of B cell–depleting antibody Rituxan rituximab in a Phase II/III lupus trial. Long-lived plasma cells have remained therapeutically intractable.[3]

A paper in *Nature Medicine* indicates that Velcade bortezomib might offer a therapeutic option in lupus, as the small molecule proteasome inhibitor depletes both short- and long-lived plasma cells. Academic and company researchers are concerned about potential side effects, however, because a proteasome inhibitor may not be sufficiently selective to target only autoantibody-producing plasma cells.

Lupus is a chronic autoimmune disease characterized by excessive production of autoantibodies that can cause severe damage to the skin, kidneys, nervous system, blood and other organs. Strategies to treat the disease have aimed to suppress or at least significantly reduce autoantibody levels by targeting subpopulations of the B cell family (*see* **Table 1, "Lupus pipeline"**). For example, the monoclonal antibody Rituxan targets the CD20 receptor, which is expressed on mature and memory B cells, but not on plasma cells.

Because plasma cells have few if any cell surface markers in common with mature and memory B cells, targeting them with therapies like Rituxan is unlikely. Moreover, it is still unclear where in the plasma cell developmental pathway intervention would best prevent autoantibody production.[4] Consequently, there are as yet no therapies in the clinic that directly target either short- or long-lived plasma cells.

The *Nature Medicine* article builds on previous work by two different labs. Rudolf Manz and colleagues have shown that autoantibody-secreting plasma cells contribute to autoimmune disease in a mouse model of lupus.[5] Reinhard Voll and colleagues have reported that high rates of antibody production sensitized multiple myeloma cells to bortezomib.[6]

In the latest paper, Voll and Manz jointly investigated whether bortezomib's efficacy against malignant plasma cells translated into efficacy against autoantibody-producing plasma cells in lupus.[7]

In initial experiments in a murine model of lupus nephritis, bortezomib targeted and depleted plasma cells. The researchers found that this depletion correlated with improvements on key disease endpoints. In the mice, twice-weekly administration of bortezomib significantly

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lowered autoantibody levels and prolonged survival compared with what was seen in mice that received saline buffer control ($p<0.001$ for both).

In a second murine model of aggressive lupus with both kidney and skin involvement, bortezomib again significantly lowered autoantibody levels and increased survival compared with what was seen in controls ($p=0.04$ and $p=0.03$, respectively).

Manz told *SciBX* that the continuous production of autoantibodies in systemic lupus erythematosus is due to two mechanisms. The first of these is chronic B cell activation that leads to the formation of short-lived plasma cells, whereas the second is survival of long-lived plasma cells, he said.

Thus, even in patients who respond well to conventional lupus therapies, said Manz, "persistent but low-level autoantibody production by long-lived plasma cells is likely contributing to increased risk for relapse." In patients who respond poorly to conventional therapy, he said, "long-lived plasma cells might be the major source of autoantibodies."

Manz, a researcher in the Department of Humoral Immunology at the **German Arthritis Research Center**, therefore expects that bortezomib could be useful in treating lupus patients who respond poorly or not at all to immunosuppressants or B cell–depletion therapy.

Not so fast

Velcade is marketed by **Millennium Pharmaceuticals Inc.**, a subsidiary of **Takeda Pharmaceutical Co.**, and **Johnson & Johnson** to treat mantle cell lymphoma and multiple myeloma (MM). According to the label, the recommended dose of the drug is 1.3 mg/m^2 delivered via i.v. twice a week for two weeks followed by a ten-day rest period. The drug's side effects include peripheral neuropathy, pulmonary disorders and neutropenia.

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Table 1. Lupus pipeline. A number of compounds for lupus are in late-stage development, with at least 11 products in Phase II or Phase III testing.

Company	Product	Description	Indication	Status
Genelabs Technologies Inc. (NASDAQ: GNLB)/**Genovate Biotechnology Co. Ltd./Teva Pharmaceutical Industries Ltd.** (NASDAQ:TEVA)/ **Watson Pharmaceuticals Inc.** (NYSE:WPI)	Prestara prasterone	Synthetic dehydroepiandrosterone hormone	Systemic lupus erythematosus (SLE)	Phase III
Human Genome Sciences Inc. (NASDAQ:HGSI)/ **GlaxoSmithKline plc** (LSE:GSK; NYSE:GSK)	Belimumab (LymphoStat-B)	Human mAb against B lymphocyte stimulator protein	SLE	Phase III
Galenica AG (SWX:GALN)/**Roche** (SWX:ROG)	CellCept mycophenolate mofetil	Reversible inhibitor of inosine monophosphate dehydrogenase	Lupus nephritis	Phase III
Biogen Idec Inc. (NASDAQ:BIIB)/**Genentech Inc.** (NYSE:DNA)/Roche	Rituxan rituximab	Chimeric mAb against CD20 antigen	Lupus nephritis	Phase III
Biogen Idec/Genentech/Roche	Ocrelizumab	Second-generation humanized mAb against CD20	Lupus nephritis	Phase III
La Jolla Pharmaceutical Co. (NASDAQ:LJPC)	Riquent abetimus	Carrier plus four double-stranded oligonucleotides that bind to anti–double-stranded DNA antibodies on B cells	Lupus nephritis	Phase III
Immunomedics Inc. (NASDAQ:IMMU)/**UCB Group** (Euronext:UCB)	Epratuzumab	Humanized mAb against CD22	SLE	Phase III
Bristol-Myers Squibb Co. (NYSE:BMY)	Orencia abatacept	Cytotoxic T lymphocyte antigen 4–Ig fusion protein	Lupus nephritis	Phase II/III
ZymoGenetics Inc. (NASDAQ:ZGEN)/**Merck KGaA** (Xetra:MRK)	Atacicept	Soluble fusion protein containing the extracellular portion of transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) linked to Fc	SLE; lupus nephritis	Phase II/III
Astion Pharma A/S	ASF-1096	0.5% cream formulation of R-enantiomer of salbutamol (albuterol)	Cutaneous lupus erythematosus (CLE)	Phase II
ImmuPharma plc (LSE:IMM)	Lupuzor (IPP-201101)	Peptide fragment analog including residues 131–151 of the spliceosomal U1-70K protein with a phosphorylated serine at position 140	SLE	Phase II

In both cancer and lupus, Velcade is eliminating plasma cells. In the former case, these cells are malignant, but in lupus, none are malignant and only a fraction produce autoantibodies. Nonetheless, the *Nature Medicine* paper notes that bortezomib depleted both autoantibody-producing and normal plasma cells "without causing overt toxic effects in mice."

Moreover, the authors wrote, other immune effector cells, such as resting B and T cells, monocytes and dendritic cells "were not affected or were moderately affected particularly after prolonged treatment, indicating a certain level of selectivity for plasma cells."

Nevertheless, lupus researchers polled by *SciBX* emphasized the need for additional preclinical work before putting bortezomib into patients.

Prior to putting the drug into the clinic for lupus, Voll told *SciBX* that "it would be helpful to find a suitable combination therapy and to optimize the treatment schedule in animals before starting a clinical trial in antibody-mediated autoimmune diseases."

Voll is a researcher in the Department of Internal Medicine and the Institute of Clinical Immunology at the **University of Erlangen-Nuremberg**.

Noel Rose, professor and director of the Center for Autoimmune Disease Research at the **Johns Hopkins Bloomberg School of Public Health**, noted that bortezomib's specificity for normal—as opposed to malignant—plasma cells was unexpected. "The downside is that a proteasome inhibitor is predicted to have side effects in other rapidly proliferating cell types such as the gastrointestinal tract," he said. "These potential toxicities should be carefully addressed in lupus animal models before testing bortezomib in patients."

Tony Manning, VP of inflammation and autoimmune diseases at **Biogen Idec Inc.**, also expressed concerns about treating lupus with bortezomib.

In addition to potential off-target tissue toxicity, Manning cautioned that bortezomib "could also deplete protective B cells, which might increase risk for opportunistic infection during long-term treatment. The authors also showed suppression of lymphocyte numbers in their studies. Thus, there's the concern that the dosing window could be too narrow to use the drug in humans without serious risk of adverse events."

Although plasma cells are being targeted in both lupus and multiple myeloma, Manning noted that "these are still two very different indications and patient populations."

Biogen Idec is exploring strategies that deplete plasma cells in lupus and other autoimmune diseases.

"We have two preclinical programs of note in this area," said Manning. "One of them is an antibody therapy that targets an undisclosed molecule that we believe is restricted to short-lived plasma cells. We are exploring the hypothesis that these cells are in large part responsible for the production of pathogenic autoantibodies that drive diseases such as lupus. The other is a small molecule inhibitor of the molecular chaperone Hsp90 [heat shock protein 90] that triggers the unfolded protein response in plasma cells, ultimately causing apoptosis."

"The *Nature Medicine* paper illustrates an awareness among lupus researchers that plasma cells probably play a critical role in disease pathophysiology."

—Matthew Linnik, La Jolla Pharmaceutical Co.

Biogen Idec also has multiple Phase III trials testing Rituxan and ocrelizumab, a second-generation anti-CD20 mAb, in general lupus and in lupus nephritis. A trial of Rituxan in lupus nephritis is ongoing, and initial data from the two ocrelizumab trials are expected at the end of 2009, Manning said.

Rituxan is marketed by **Genentech Inc.**, Biogen Idec and **Roche** to treat non-Hodgkin's lymphoma (NHL) and rheumatoid arthritis (RA). The three companies are co-developing ocrelizumab.

Matthew Linnik, EVP and CSO of **La Jolla Pharmaceutical Co.**, told *SciBX*, "The toxic nature of proteasome inhibitor therapy could mean that side effects like peripheral neuropathy and neutropenia limit its use to catastrophic forms of lupus that are associated with conditions like antiphospholipid syndrome, where autoantibodies to cell membrane phospholipids cause massive clotting in the circulation and organ systems."

Nevertheless, "the *Nature Medicine* paper illustrates an awareness among lupus researchers that plasma cells probably play a critical role in disease pathophysiology," said Linnik. "For very long periods of time, a plasma cell can occupy a survival niche in the bone marrow where it steadily pumps out antibodies or, in the case of lupus, autoantibodies, which help sustain disease. Moreover, these cells don't express CD20 on their surface and are therefore refractory to Rituxan and similar therapies that target B cells."

The question is how to exploit the plasma cell findings in the *Nature Medicine* paper while avoiding bortezomib's documented side effects in cancer patients.

"One obvious strategy would be to identify components unique to long-lived plasma cells and target those," said Linnik. "Unfortunately, these cells have historically been quite difficult to culture and work with. Nevertheless, a plasma cell–targeted therapy, if it existed, would probably have a reduced side-effect profile compared with a drug like bortezomib, which targets a pathway common to many cell types."

La Jolla's Riquent abetimus, a carrier molecule linked to four double-stranded oligonucleotides that binds anti–double-stranded DNA autoantibodies on the surface of memory B cells, is in Phase III testing to treat lupus and lupus nephritis and to prevent renal flares.

"Autoantibodies against double-stranded DNA contribute to lupus nephritis and are also present in many nonrenal forms of lupus," said Linnik. "Abetimus is designed to specifically suppress activation of the B cell subpopulation that produces autoantibodies against double-stranded DNA without compromising the important immune function of the remaining B cells."

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T time for tumors

By Lev Osherovich, Senior Writer

No therapeutic cancer vaccine has yet shown sufficient efficacy to be approved in the U.S., in part because of the complex immune response to vaccination. A study in *Cancer Cell* helps refine the tactics for developing pancreatic cancer vaccines, showing that local activity by tumor-seeking helper T cells can retard—or promote—tumor development.[1]

The study, conducted by a team led by Martin Röcken, professor of dermatology at **Eberhard Karls University**, could help cancer vaccine makers design therapies that direct T cells to the right place and elicit the right kind of immune response.

The group used an established mouse model in which overexpression of a viral oncoprotein called T antigen (TAG) promotes islet cell adenomas and, eventually, carcinomas in the pancreas.[2] Such tumors develop extensive vascular structures that facilitate tumor growth.

The researchers raised helper T cells that recognized a TAG fragment in cell culture. The T cells were labeled with a fluorescent dye and injected into mice. Within days, the TAG-seeking T cells migrated to pancreatic lymph nodes in TAG-expressing mice but not in wild-type controls.

Once on the scene, the T cells prevented both the appearance of new tumors and the expansion of existing ones beyond the adenoma stage. Compared with mock-treated controls, mice that received TAG-specific T cell transfusions had fewer and smaller pancreatic tumors with less extensive vascularization.

T cell–treated mice, including those treated after tumors started to form, lived longer than mock-treated controls.

"We show that T cells can induce tumor dormancy," Röcken told *SciBX*.

Playing TAG with tumors

According to conventional wisdom, helper T cells act indirectly in cancer vaccines by secreting cytokines that prompt cytotoxic T cells to attack tumors. This principle is the basis of several therapeutic cancer vaccines.[3]

However, Röcken's team found that mice lacking most of their cytotoxic T cells still could benefit from TAG-specific helper T cells. Although the pancreatic tumors of these T cell–treated mice showed no signs of apoptosis, which would be indicative of cytotoxic T cell attack, the researchers did see a decrease in the pancreatic incorporation of bromodeoxyuridine (BrdU) compared with that seen in wild-type controls. BrdU labels newly replicated DNA in proliferating tumors.

Thus, the helper T cell–based treatment did not kill tumor cells but, instead, arrested their growth.

Röcken's team next asked how proinflammatory cytokines such as interferon-γ (IFN-γ) influence homing and tumor restriction by TAG-specific T cells.[4] In mice depleted of IFN-γ, Röcken's team found that T cells still migrated to the pancreas, but they promoted tumor growth instead of preventing it. IFN-γ-depleted mice that received tumor-targeting T cells had larger and more highly vascularized tumors than untreated IFN-γ-depleted mice.

The team got similar results in mice lacking tumor necrosis factor receptor 1 (TNFR1), a target of the inflammatory cytokine tumor necrosis factor-α (TNF-α). Even without added T cells, TNFR1 mutation shortened the survival of TAG-expressing mice compared with that of wild-type controls. TNFR1-deficient mice that received T cell therapy had even bigger tumors with more extensive BrdU incorporation and did not survive as long as untreated controls.

These findings suggest that in the absence of TNF signaling, T cells exacerbate cancer progression.

Fine lines

Röcken told *SciBX* that the study illustrates the fine line between help and harm in cancer vaccine development.

"If you have the right combination of cytokines, it works, but if you generate the wrong cytokine pattern, T cells can actually enhance tumor growth," he said.

> **"We show that T cells can induce tumor dormancy."**
> ***—Martin Röcken, Eberhard Karls University***

Röcken noted that TNFR1 experiments show that the outcome of T cell treatment is influenced by at least three types of cells: the T cells themselves, the tumor cells and the surrounding noncancerous cells that develop into vascular tissue in response to signals from tumor cells.

In the TNFR1 mutants, "the T cells are still functioning normally," said Röcken. "The defect is in the microenvironment or in the tumor itself," although the precise cells responding to TNF signaling remain unknown.

Ruth Ganss, associate professor at the **Western Australian Institute for Medical Research**, told *SciBX* that the study reveals many new questions about which cells mediate the IFN-γ and TNF-α effects and how vasculogenic cells in the tumor environment respond to nearby T cells.

Another outstanding question is whether T cells change their portfolio of cytokines when exposed to a tumor microenvironment. Röcken told *SciBX* that in the absence of IFN-γ and TNFR1, the T helper type 1 (Th1) cells used in the study may behave more like T helper type 2 (Th2) cells.

Th2 cells have been associated with negative outcomes in cancer vaccine trials, perhaps because of an altered balance of pro- and anti-inflammatory cytokines.[5]

Zena Werb, a professor of anatomy at the **University of California, San Francisco**, told *SciBX* that the experiments with IFN-γ suggest that immune cells in the surrounding microenvironment, such as macrophages, are critical for T cell control of tumors.

"It is already known that interferon-γ or interferon-γ-producing T cells protect against malignancies," said Werb. "This study explains how and also shows that it can be subverted."

Werb suggested that the next step would be to administer IFN-γ or "short treatments of TNF" to tumor sites to see if these cytokines are sufficient to suppress tumor growth.

Along these lines, Röcken cited evidence that IFN-γ and TNFR1 have been shown to work together to suppress tumor growth in a mouse model of spleen cancer.[6] However, both Röcken and Robert

(Continues on p. 6)

SK1-I is the limit in AML

By Lauren Martz, Staff Writer

A paper published in *Blood* suggests that going upstream of the known cancer target sphingosine 1-phosphate could be a better approach than hitting site-1 protease (S1P) itself. By specifically hitting one of the two sphingosine kinase isoenzymes that produce S1P, researchers at **Virginia Commonwealth University School of Medicine** think they have a small molecule cancer lead—dubbed SK1-I—with broader effects than S1P inhibitors and more selective effects than compounds that inhibit both isoenzymes.[1] The upshot could be a compound for acute myelogenous leukemia with increased efficacy and fewer off-target effects.

S1P is a sphingolipid mediator involved in calcium metabolism, cytoskeletal reorganization, immune cell trafficking, cell growth, differentiation, motility, angiogenesis and survival.[2] It is produced by a pair of sphingosine kinase isoenzymes—sphingosine kinase 1 (SphK1) and SphK2—that have opposite functions.[3]

SphK1 promotes proliferation and survival by catalyzing a reaction that produces S1P and depletes levels of ceramide and sphingosine, which are proapoptotic precursors of S1P. Studies have shown that SphK1 is upregulated in cancer cells.[4]

In the *Blood* paper, Sarah Spiegel and colleagues synthesized (2*R*,3*S*,4*E*)-*N*-methyl-5-(4′-pentylphenyl)-2-aminopent-4-ene-1,3-diol, called SK1-I, which they said is the first water-soluble inhibitor specific for SphK1. Spiegel is corresponding author on the paper and a professor of biochemistry, molecular biology and oncology at Virginia Commonwealth.

In human leukemia cells, SK1-I increased the ratio of ceramide to S1P, which resulted in decreased cell growth and increased apoptosis. SK1-I reduced tumor volume by about 50% in xenograft mice.

(Continues on p. 7)

(Continued from **"T time for tumors,"** p. 5)

Burns, CEO of **Immunox Inc.**, noted that neither IFN-γ nor TNF-α monotherapy has been especially successful in cancer trials.

Indeed, Ganss told *SciBX* that "since dosing and timing of cytokines is so critical, it will be difficult to design a cytokine therapy that can mimic the complex immune response described in the *Cancer Cell* paper."

T trials

Burns said the Röcken study illustrates that inflammatory cytokines such as IFN-γ and TNF-α are needed to prime the immune response to tumors and thus could be important elements of vaccine adjuvant strategies.

Immunox is developing adjuvants for therapeutic and prophylactic vaccines. Burns was formerly CEO of cancer vaccine company Celldex Therapeutics Inc., now part of **Avant Immunotherapeutics Inc.**

"We know that the immune system struggles to recognize a tumor in the absence of some sort of alerting signal," said Burns. Without inflammatory cytokines, "the alternative could be a sort of subversion" of normal immunological activity by the tumors, leading to enhanced tumor growth.

Burns cautioned that differences in the immunological behavior of mouse and human tumors make it difficult to predict whether the Röcken study will be helpful in developing cancer vaccines.

Kristen Hege, VP of clinical research at cancer vaccine company **Cell Genesys Inc.**, said that Röcken's study provides a new model for probing interactions between the immune system and tumor microenvironment in angiogenesis.

"This area has been historically underappreciated," she said.

Hege said that although previous studies suggested that therapeutic vaccines could promote tumor dormancy, this study provides the first mechanistic insights into the process.

The findings also increase the toolbox of immunological tricks that can be deployed by cancer vaccines.

Early cancer vaccine strategies used helper T cell responses primarily for "regulating or optimizing a cytotoxic T cell response" Hege noted. The Röcken study "raises in my mind the possibility of combining immunotherapy with antiangiogenic effects" mediated by IFN-γ or other helper T cell–derived cytokines.

Cell Genesys uses its GVAX cancer vaccine platform to target a range of tumor types. The company's lead vaccine for prostate cancer consists of irradiated tumor cells supplemented with granulocyte/macrophage colony-stimulating factor (GM-CSF), a dendritic cell adjuvant that enhances acquired immunity to tumor factors.[7]

GVAX vaccine for prostate cancer is in Phase III trials. GVAX vaccine for pancreatic cancer is in Phase II trials.

Röcken said the work in this study was not patented, but that he was "open to working with industry" to further develop the technology.

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Western Australian Institute for Medical Research, UWA Centre for Medical Research, Perth, Australia

Moreover, the compound induced apoptosis in leukemic blasts from two human acute myelogenous leukemia (AML) patients while sparing normal leukocytes.

The only published SphK inhibitors "inhibit both SphK1 and SphK2 and are very toxic," because they also are blocking SphK2, Spiegel told *SciBX*.

Nonspecific SphK inhibition is dangerous because it causes a deficiency in S1P, which has such a broad range of cellular effects. Knockout of both SphK isoforms in mice disrupted normal neurogenesis and angiogenesis and was embryonically lethal.[5]

> **"The major problem of the field is that the 3D structure of the kinase is not described. Until this is achieved, no really specific inhibitors will be found."**
>
> ***—Olivier Cuvillier, Centre National de la Recherche Scientifique (CNRS)***

A key to eliminating cross-reactivity with SphK2 was designing SK1-I such that the molecule does not bind to its target's ATP-binding pocket. The binding pocket is a well-conserved region of many protein kinases, including SphK1 and SphK2.

Better than blocking S1P

In addition to SK1-I's potential for fewer side effects than pan-SphK inhibitors, the compound could have improved efficacy compared with S1P antagonists.

According to the *Blood* paper's authors, their molecule decreases "pro-growth and survival S1P while simultaneously increasing its precursor, the pro-apoptotic ceramide. Hence, SK1-I integrates multiple molecular therapeutic targets in leukemia."

Roger Sabbadini, VP and CSO of **Lpath Inc.**, told *SciBX* that SK1-I might also have the potential to overcome chemotherapy resistance. He noted that SphK1 can prevent the apoptotic effects of chemotherapy drugs and that levels of SphK1 indeed correlate with resistance to chemotherapy.

Lpath is developing Asonep, a monoclonal antibody against S1P that is in Phase I testing for cancer.

Although Sabbadini acknowledged the benefits of specifically targeting SphK1, he cautioned that blocking the enzyme might not be as effective as targeting its prosurvival product, S1P. One reason, he said, is that targeting a kinase inside the cell (SphK1) is more difficult than targeting the extracellular sphingolipid (S1P).

Asonep "binds to and neutralizes S1P in the extracellular environments," Sabbadini noted. In addition, he said, "the major problem with targeting SphK1 is that there are other ways to make S1P. Our approach doesn't care where the S1P comes from."

Thus, Sabbadini said a potential next step for SK1-I is to "evaluate extracellular levels of S1P to see how much of the burden is eliminated by inhibiting its production. This could be demonstrated in preclinical mouse models."

Spiegel countered that the antibody approach only inhibits extracellular S1P. Her lab and others have shown that internal S1P also promotes growth and inhibits death of cancer cells.[6] "Our inhibitor, by blocking production of S1P, would block this internal action of S1P as well as its external actions," she told *SciBX*.

Olivier Cuvillier, head of the Sphingolipids & Cancer Research Lab at the Institute of Pharmacology and Structural Biology of the **Centre National de la Recherche Scientifique** (CNRS), agreed. He said the benefits of the approach published in *Blood* are that both the intracellular and possibly extracellular effects of S1P production are blocked.

Among other next steps, Sabbadini said, "we need to know the pharmacokinetics and pharmacodynamics, half-life, biodistribution and druggability," of SK1-I. The authors of the *Blood* paper "need to initially put this compound into rodents, then nonhuman primates to establish a therapeutic index."

"We have already shown it can be administered to mice at effective doses without any obvious toxic effects," said Speigel. "After more complete toxicology and pharmacokinetics data, we hope to make modifications to the structure that will increase its potency."

Structure improvements could be challenging, noted Cuvillier, because "the major problem of the field is that the 3D structure of the kinase is not described. Until this is achieved, no really specific inhibitors will be found."

Eventually, SK1-I could find a home in a cocktail of therapeutics for AML.

Lina Obeid, professor of medicine and of biochemistry and molecular biology at the **Medical University of South Carolina**, noted that cancers usually disrupt multiple parts of the pathways that regulate cell growth and development. Thus, she said, inhibitors of SphK1 have potential in combination with therapies targeting other parts of the sphingosine pathway, such as S1P.

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Lpath Inc. (OTCBB:LPTN), San Diego, Calif.
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Antagonizing hepatitis

By Brian Moy, Staff Writer

A paper published in *Nature Medicine* suggests that serotonin antagonists could be useful for accelerating the clearance of potentially any type of hepatitis infection and minimizing liver cell damage. The finding could open up new therapeutic areas for biotech and pharma companies with serotonin antagonists on the market or in late-stage development for neurological conditions. The challenge will be finding antagonists with specificities that make it possible to treat hepatitis without interfering with serotonin's other roles in the liver.

Researchers at the **University Hospital Zurich** showed that the release of endogenous platelet-derived vasoactive serotonin in the liver delayed the entry of activated virus-specific $CD8^+$ T cells into the livers of wild-type mice infected with lymphocytic choriomeningitis virus (LCMV), a model of human hepatitis. Virus-specific T cells play a major role in attacking chronic hepatitis.

Indeed, in the model, increased platelet recruitment to the liver correlated with lower sinusoidal microcirculation, delayed virus removal and greater liver cell damage. Low hepatic sinusoidal microcirculation—blood flow through the liver—is responsible for delaying infiltration of activated $CD8^+$ T cells, which can result in the persistence of infection.

Infected mice treated with exogenous serotonin also had delayed entry of $CD8^+$ T cells into the liver. The opposite was seen in serotonin-deficient mice. In those mice, the lack of platelet-derived serotonin after LCMV infection resulted in better viral clearance and less liver cell damage than was seen in wild-type mice.[1]

"We were initially investigating the role of serotonin following virus-induced liver cell damage, and, to our surprise, absence of serotonin prevented liver cell damage during virus-induced hepatitis," said Karl Lang, corresponding author on the paper. Lang, now a research assistant at the **University of Toronto**, is investigating the role of serotonin in later stages of hepatitis.

He said there are two possible approaches for developing serotonin antagonists for hepatitis: testing known serotonin antagonists in hepatitis patients or screening for new serotonin antagonists that do not cross the blood-brain barrier and analyzing these compounds for their ability to clear hepatitis in animal models (*see* **Table 1, "Targeting 5-HT_2"**).

Table 1. Targeting 5-HT_2. Previous studies have identified the serotonin 5-HT_{2A} and 5-HT_{2B} receptors, which are expressed by hepatic stellate cells, as regulators of sinusoidal blood flow.[3] In hepatitis, decreased sinusoidal microcirculation caused by platelet-derived vasoactive serotonin delays removal of the virus and increases liver cell damage. Thus, existing antagonists targeting the 5-HT_2 family of serotonin receptors could have potential utility in accelerating clearance of hepatitis infection. However, the antagonist must be specific enough to prevent the reduction in sinusoidal blood flow without interfering with serotonin's other roles in the liver such as regulating hepatic stellate cell function, hepatocyte growth and bile duct epithelial cell proliferation. Below are selected antagonists or inverse agonists that target serotonin (5-HT_2) receptors in Phase III or on the market. Some of the compounds also target other proteins such as dopamine receptors.

Company	Product	Indication	Lead status
Eli Lilly and Co. (NYSE:LLY)	Zyprexa olanzapine	Bipolar disorder; schizophrenia	Marketed
Novartis AG (NYSE:NVS; SWX:NOVN)	Clozaril clozapine	Schizophrenia	Marketed
Pfizer Inc. (NYSE:PFE)	Geodon ziprasidone	Bipolar disorder, schizophrenia	Marketed
AstraZeneca plc (LSE:AZN; NYSE:AZN)	Seroquel/Seroquel XR quetiapine	Bipolar disorder, schizophrenia	Marketed
H. Lundbeck A/S (CSE:LUN)	Serdolect sertindole	Bipolar disorder, schizophrenia	Marketed
Johnson & Johnson (NYSE:JNJ)/**Alkermes Inc.** (NASDAQ:ALKS)	Risperdal/Risperdal Consta risperidone	Bipolar disorder, schizophrenia	Marketed
Vanda Pharmaceuticals Inc. (NASDAQ:VNDA)	Fanapta iloperidone	Schizophrenia	7/27 PDUFA date
Acadia Pharmaceuticals Inc. (NASDAQ:ACAD)	Pimavanserin (ACP-103)	Parkinson's disease psychosis	Phase III
Boehringer Ingelheim GmbH	Flibanserin	Hypoactive sexual desire disorder (HSDD)	Phase III
Dainippon Sumitomo Pharma Co. Ltd. (Tokyo:4506; Osaka:4506)/**Merck & Co. Inc.** (NYSE:MRK)	Lurasidone (SM-13496)	Schizophrenia	Phase III
Mitsubishi Tanabe Pharma Corp. (Tokyo:4508; Osaka:4508)	Anplag sarpogrelate	Prevent recurrence of cerebral infarction	Phase III
sanofi-aventis Group (Euronext:SAN; NYSE:SNY)	Eplivanserin	Insomnia	Phase III
sanofi-aventis	Volinanserin	Insomnia	Phase III
Schering-Plough Corp. (NYSE:SGP)	Org 50081	Insomnia	Phase III

According to Lang, potential side effects of serotonin antagonists that do cross the blood-brain barrier include nausea, drowsiness, dizziness, changes in appetite, weight loss or weight gain and sexual dysfunction.

Serotonin's receptors

With either approach it is likely that specificity will be the key consideration. The reason is that broadly antagonizing serotonin in the liver could also disrupt serotonin's role in regulating hepatic stellate cell function, hepatocyte growth and bile duct epithelial cell proliferation.

> **"Because there are so many serotonin receptors, further research needs to determine which specific receptors are responsive to targeting in patients with hepatitis."**
>
> ***—Michael Kauffman, Epix Pharmaceuticals Inc.***

Previous studies have shown that platelet-derived serotonin regulates liver regeneration.[2]

According to Derek Mann, a professor of hepatology at **Newcastle University**, "pan-blockade of serotonin would be problematic, as serotonin is required for liver regeneration in response to injury."

On the other hand, he said, "blocking specific receptors that drive inflammatory and fibrogenic responses could enable the negative effects of serotonin to be prevented while retaining the beneficial properties of serotonin."

Michael Kauffman, CEO of **Epix Pharmaceuticals Inc.**, told *SciBX* that "because there are so many serotonin receptors, further research needs to determine which specific receptors are responsive to targeting in patients with hepatitis." To achieve this, he said that "preclinical testing of known serotonin antagonists in animal models of hepatitis could identify which receptors play a role in disease."

Kauffman added that "once the appropriate receptors are identified, it will be useful to take liver biopsies from patients with hepatitis and stain them for specific serotonin receptors." At that point, he said, "we can move into clinical testing using the proper antagonists."

Around mid-year, Epix plans to start a Phase II trial of PRX-08066, a selective serotonin (5-HT_{2B}) receptor antagonist, to treat pulmonary hypertension associated with chronic obstructive pulmonary disease (COPD). Also this year, Epix plans to begin a Phase IIa trial of PRX-07034, a selective serotonin (5-HT_{6}) receptor antagonist, to treat cognitive impairment associated with schizophrenia.

Richard Ruddell, a research officer in the Hepatic Fibrosis Group of the **Queensland Institute of Medical Research** and an adjunct lecturer at the **University of Queensland**, said that the *Nature Medicine* paper "does not hint at the receptor type mediating the effects of serotonin. Nonspecific antagonists may inhibit hepatic regeneration stimulated by serotonin as well as increase sinusoidal blood flow."

Ruddell thinks the best results will come from hitting the 5-HT_{2A} and 5-HT_{2B} receptors. He and other researchers have identified those two receptors, and other serotonin receptor family members within the liver, as being particularly relevant to sinusoidal blood flow.

The 5-HT_{2A} and 5-HT_{2B} receptors, which are expressed by hepatic stellate cells, are thought to regulate sinusoidal blood flow by regulating the contractility of the cells.[3]

Additionally, Ruddell is investigating where serotonin receptors are located within the liver and how they are expressed during disease.

"At present, there are no ligands that will only bind to one receptor and not the other," he noted. "If you can find an antagonist that is selective enough to prevent only the reduction in sinusoidal microcirculation invoked by serotonin within the liver, there may be a potential role for serotonin antagonists in treating hepatitis."

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Queensland Institute of Medical Research, Brisbane, Australia
University of Queensland, Brisbane, Australia
University Hospital Zurich, Zurich, Switzerland
University of Toronto, Toronto, Ontario, Canada

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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 40 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

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

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Autoimmune disease				
Autoimmune disease	Cytoplasmic tail of CD45 (ct-CD45)	A study in cell culture suggests that ct-CD45 could be useful for treating autoimmune disorders. CD45 is proteolytically processed during the activation of human monocytes and granuloctyes by fungal stimuli, and the resulting fragment lowered dendritic cell– and CD3 antibody-induced T cell proliferation compared with that seen in untreated controls. The inhibitory effect of ct-CD45 was comparable to that found with IL-10, a standard inhibitory factor for T cells. Next steps include identifying the receptor for ct-CD45 and evaluating the peptide as an immunosuppressive agent in a murine model of rheumatoid arthritis (RA).	Not patented; available for licensing from the Medical University of Vienna	Kirchberger, S. *et al. Blood*; published online May 29, 2008; doi:10.1182/blood-2008-02-138131 **Contact:** Johannes Stockl, Medical University of Vienna, Vienna, Austria e-mail: johannes.stoeckl@meduniwien.ac.at
Autoimmune disease	Fc γ-receptor IIa (FCGR2A; CD32); Fc γ-receptor IIIb (FCGR3B; CD16)	A study suggests that antagonizing FCGR2A on neutrophils may help treat or prevent tissue damage associated with inflammation in autoimmune disease. Mice engineered to express either human FCGR2A or FCGR2A and FCGR3B on neutrophils were protected from IgG-mediated glomerulonephritis and reverse passive Arthus. Mice expressing FCGR2A also had higher levels of edema and neutrophil accumulation in these disease models than wild-type mice. Next steps include developing strategies for neutrophil-selective inhibition of FCGR2A in autoimmune disease. Trillium Therapeutics Inc. has an FCGR2A antagonist in development to treat autoimmune disorders.	Not patented; available for licensing through Brigham and Women's Hospital	Tsuboi, N. *et al. Immunity*; published online June 5, 2008; doi:10.1016/j.immuni.2008.04.013 **Contact:** Tanya N. Mayadas, Brigham and Women's Hospital and Harvard Medical School, Boston, Mass. e-mail: tmayadas@rics.bwh.harvard.edu
Lupus	Proteasome	A study in mice suggests that Velcade bortezomib could be used for treating lupus and other autoimmune diseases. In the NZB/W F1 mouse model of lupus nephritis, twice-weekly bortezomib significantly lowered autoantibody levels and prolonged survival compared with what was seen in mice that received saline buffer control ($p<0.001$ for both). In the MRL/lpr murine model of aggressive lupus with kidney and skin involvement, bortezomib significantly lowered autoantibody levels and increased survival compared with what was seen in untreated controls ($p=0.04$ and $p=0.03$, respectively). The proteasome inhibitor depleted both short- and long-lived plasma cells that produce autoantibodies. Next steps include finding a combination therapy and treatment schedule in animals and running clinical trials in lupus. Millennium Pharmaceuticals Inc., a subsidiary of Takeda Pharmaceutical Co. Ltd., and Johnson & Johnson market Velcade bortezomib to treat multiple myeloma (MM) and mantle cell lymphoma. NPI-0052, a proteasome inhibitor from Nereus Pharmaceuticals Inc., is in Phase I testing to treat cancer. (*See* **Moving Velcade into lupus, page 1**.)	Patent application filed in Germany for use of proteasome inhibitors to deplete long-lived plasma cells; available for licensing	Neubert, K. *et al. Nat. Med.*; published online June 8, 2008; doi:10.1038/nm1763 **Contact:** Reinhard Voll, University of Erlangen-Nuremberg, Erlangen, Germany e-mail: rvoll@molmed.uni-erlangen.de

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer				
Acute myelogenous leukemia (AML), leukemia	Sphingosine kinase 1 (SphK1)	Studies in cancer cells and in mice suggest that inhibitors specific for SphK1 could potentially be used to treat cancer. The small molecule SphK1 inhibitor SK1-I blocked cell growth and promoted apoptosis in U937 leukemia cells and Jurkat T cells. SK1-I treatment of xenograft mouse models lowered U937 tumor volume by about 50% compared with that in saline-treated controls. The inhibitor also promoted apoptosis in leukemic blasts from two AML patients while sparing normal leukocytes. Additional preclinical toxicology studies are necessary before SK1-I can be tested in humans. Lexicon Pharmaceuticals Inc., Lpath Inc. and Mitsubishi Tanabe Pharma Corp. have separate compounds that modulate site-1 protease, a downstream target of SphK1, in clinical and preclinical development to treat autoimmune diseases and cancer. (*See* **SK1-I is the limit in AML, page 6**.)	Provisional patent issued for the inhibitor; licensing status undisclosed	Paugh, B. *et al. Blood*; published online May 29, 2008; doi:10.1182/blood-2008-02-138958 **Contact:** Sarah Spiegel, Virginia Commonwealth University, Richmond, Va. e-mail: sspiegel@vcu.edu
Acute myelogenous leukemia (AML); leukemia; multiple myeloma (MM)	Cyclin D1 (CCND1; BCL1); cyclin D2 (CCND2); cyclin D3 (CCND3)	Studies in cell lines and mice suggest the small molecule D-cyclin inhibitor cyproheptadine could be useful for treating AML and other hematological cancers. In myeloma and leukemia cell lines, the compound induced apoptosis compared with what was seen in untreated controls. In mouse models of myeloma and leukemia, the compound inhibited tumor growth, whereas minimal toxicity was observed in normal hematopoietic cells. Next steps include developing an oral formulation of the compound for use in clinical trials and identifying new compounds that target D-cyclins. Cyproheptadine is marketed by Merck & Co. Inc. under the name Periactin to treat allergies and hay fever. The compound is also used as an appetite stimulant in cats. LOR-253, a small molecule downregulator of the cyclin D oncogene, is in preclinical testing to treat cancer.	Patent application filed for use of cyproheptadine to treat leukemia and myeloma; available for licensing through the University Health Network	Mao, X. *et al. Blood*; published online May 23, 2008; doi:10.1182/blood-2008-02-142687 **Contact:** Aaron D. Schimmer, University of Toronto, Toronto, Ontario, Canada e-mail: aaron.schimmer@utoronto.ca
Endometrial cancer	Fibroblast growth factor receptor-2 (FGFR2)	A study in cell culture suggests that inhibiting mutant forms of FGFR2 may be useful for treating endometrial cancer. In three endometrial cell lines expressing mutated forms of FGFR2, treatment with the FGFR inhibitor PD173074 inhibited colony formation and lowered cell survival rates compared with those seen in cell lines expressing wild-type FGFR2. Treatment with PD173074 also inhibited phosphorylation of the FGFR2 substrate fibroblast growth factor receptor substrate 2. Links between FGFR2 mutations and cervical and squamous lung cancer, as well as congenital craniofacial malformation disorders, have been suggested in the literature. Next steps include determining the spectrum of FGFR2 mutations in a variety of cancers and determining the effect of FGFR2 mutations on cellular responses to FGFR inhibitors. The developmental status of PD173074, a compound owned by Pfizer Inc., is undisclosed.	Patent and licensing status undisclosed	Dutt, A. *et al. Proc. Natl. Acad. Sci. USA*; published online June 2, 2008; doi:10.1073/pnas.0803379105 **Contact:** Heidi Greulich, The Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, Mass. e-mail: heidig@broad.mit.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Leukemia	Wilms tumor antigen 1 (WT1)	Studies in cell lines and mice suggest that vaccines targeting WT1 could be useful for treating or preventing leukemia and other hematological cancers. In mice expressing human HLA-A2, a series of DNA vaccines encoding tetanus toxin fused to different WT1-derived peptides elicited increased T cell responses to WT1 compared with those seen when control tetanus toxin peptide was used. One of the vaccines showed activity against tumor cell lines derived from patients with acute leukemia. None of the vaccines had negative effects on hematopoietic stem cell formation. Next steps include clinical trials with the DNA vaccine in patients with chronic myeloid leukemia (CML). INNO-305, a WT1 heteroclitic peptide vaccine from Innovive Pharmaceuticals Inc., is in Phase I trials to treat acute myelogenous leukemia (AML) and both hematologic malignancies and solid tumors expressing WT1.	Patented in Europe; patent applications filed in U.S. and Japan for use of the DNA vaccine with any cancer antigen; in-licensed by Genvax Ltd. from Cancer Research Technologies Ltd.; Genvax has rights to sublicense the DNA vaccine technology	Chaise, C. *et al. Blood*; published online May 23, 2008; doi:10.1182/blood-2008-02-137695 **Contact:** Freda K. Stevenson, Southampton University Hospitals, Southampton, U.K. e-mail: fs@soton.ac.uk
Hepatocellular carcinoma (HCC)	Macrophage colony-stimulating factor (M-CSF); macrophages	Studies in human tissue samples suggest that antagonizing peritumoral M-CSF and blocking macrophage migration could help prevent recurrence or metastasis of HCC following resection. Cell distribution analyses showed that M-CSF levels and macrophage densities were significantly higher in peritumoral tissue than in intratumoral tissue ($p<0.001$). In patients with high peritumoral M-CSF levels and macrophage densities, overall survival and disease-free survival were lower than in patients who had low M-CSF levels and macrophage densities. Further studies are necessary to determine the role of peritumoral M-CSF and macrophages in hepatic cancer progression.	Not patented; unlicensed	Zhu, X.-D. *et al. J. Clin. Oncol.*; published online June 1, 2008; doi:10.1200/JCO.2007.15.6521 **Contact:** Hui-Chuan Sun, Fudan University, Shanghai, China e-mail: sun.huichuan@zs-hospital.sh.cn
Pancreatic cancer	Tumor necrosis factor receptor 1 (TNFR1); interferon-γ (IFN-γ)	A study in mice suggests that TNFR1 and IFN-γ signaling could help T cells limit tumor growth in some cancers. Mice with pancreatic cancer that received T helper 1 (Th1) cell transfer survived longer than mice that did not receive the transfer. In mice lacking IFN-γ or TNFR1, transferred Th1 cells had the opposite effect, increasing tumor vascularization and size compared with what was seen in mice that expressed IFN-γ or TNFR1. Next steps include identifying the specific cells that require IFN-γ and/or TNFR1 signaling for Th1 cells to antagonize tumor growth and developing immunotherapeutics that promote this process. Cell Genesys Inc.'s GVAX cancer vaccine is in a Phase II trial for pancreatic cancer. (*See* **T time for tumors, page 5.**)	Not patented; unavailable for licensing	Müller-Hermelink, N. *et al. Cancer Cell*; published online June 9, 2008; doi:10.1016/j.ccr.2008.04.001 **Contact:** Martin Röcken, Eberhard Karls University, Tübingen, Germany e-mail: mrocken@med.uni-tuebingen.de
Cardiovascular disease				
Heart failure	Desmin	Studies in mice suggest that preventing or disrupting desmin aggregation could be useful for preventing heart failure. Desmin is a structural protein expressed in cardiomyocytes. In a mouse model of tumor necrosis factor-α–induced cardiomyopathy, desmin formed aggregates that induced downstream apoptosis of cardiomyocytes. However, in the same mice, when cardiomyocytes expressed an aggregation-resistant desmin mutant, cardiomyocyte apoptosis decreased, left ventricular wall thinning was prevented and heart function was improved compared with what was seen in cardiomyocytes that expressed wild-type desmin. Next steps include translating the results to humans.	Not patented; licensing status undisclosed	Panagopoulou, P. *et al. J. Cell Biol.*; published online June 2, 2008; doi:10.1083/jcb.200710049 **Contact:** Yassemi Capetanaki, Academy of Athens, Athens, Greece e-mail: ycapetanaki@bioacademy.gr

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Endocrine disease				
Obesity	Leptin; anandamide	Studies in rodents suggest that exogenous leptin could be useful for treating obesity. In rats, leptin infusions into the mediobasal hypothalamus inhibited lipogenesis by suppressing endocannabinoid anandamide levels in white adipose tissue. In denervated rats, leptin infusion into the brain did not suppress lipogenesis or fat pad growth compared with that seen in rats that had intact innervation, suggesting that the sympathetic nervous system mediates the effects of centrally delivered leptin on peripheral adipose tissue. Researchers did not disclose their next steps. Metreleptin, a leptin analog from Amylin Pharmaceuticals Inc., is in Phase II testing to treat obesity. Biovitrum AB has a leptin mimetic in preclinical testing for obesity.	Not patented; unlicensed	Buettner, C. *et al. Nat. Med.*; published online May 30, 2008; doi: 10.1038/nm1775 **Contact:** Christoph Buettner, Mount Sinai School of Medicine, New York, N.Y. e-mail: christoph.buettner@mssm.edu
Infectious disease				
Fungal infections	CYP53A15	*In vitro* and *in vivo* studies suggest that targeting CYP53A15 could help treat fungal infections. *In vitro*, four phenolic compounds (isoeugenol, eugenol, vanillin and thymol) that play a role in plant resistance to fungal infection inhibited CYP53A15. Inhibition of CYP53A15 leads to increased intracellular levels of benzoic acid, which impedes fungal growth. *In vivo*, three of these compounds inhibited *Cochliobolus lunatus* growth. Next steps include solving the X-ray crystal structure of the CYP53A15 active site.	Not patented; available for licensing through the National Institute of Chemistry Slovenia, L-11: Laboratory for Biosynthesis and Biotransformation	Podobnik, B. *et al. J. Med. Chem.*; published online May 28, 2008; doi:10.1021/jm800030e **Contact:** Radovan Komel, University of Ljubljana, Ljubljana, Slovenia e-mail: radovan.komel@mf.uni-lj.si
Renal disease				
Diabetic nephropathy; renal damage	G protein–coupled receptor 91 (GPR91)	A study in mice suggests that antagonizing GPR91 may be useful for slowing or preventing progression of diabetic nephropathy. GPR91 is expressed in the kidney and binds metabolic intermediate succinate. In both healthy and diabetic mice, GPR91 knockout significantly lowered kidney renin content compared with that in controls that expressed GPR91 ($p<0.05$). Chronic secretion of high renin levels by the kidney results in hypertension, edema and diabetic nephropathy. Next steps include identifying small molecule inhibitors of GPR91 and developing a urine-based succinate assay to identify diabetic patients who are at risk of renal damage and diabetic nephropathy.	Provisional patent filed for the use of succinate as a biomarker of kidney damage; the researchers plan to make the IP available for licensing	Toma, I. *et al. J. Clin. Invest.*; published online June 5, 2008; doi:10.1172/JCI33293 **Contact:** János Peti-Peterdi, University of Southern California, Los Angeles, Calif. e-mail: petipete@usc.edu
Various				
Cancer; diabetic retinopathy	Leukemia inhibitory factor (LIF); LIF receptor	*In vitro* and *in vivo* studies suggest that targeting LIF signaling could help block angiogenesis and thus could treat cancer and diabetic retinopathy. *In vitro*, LIF inhibited hypoxia-induced VEGF expression and proliferation of cultured astrocytes. In LIF knockout mice, retinas showed increased microvessel density and VEGF expression by astrocytes in the vascularized area compared with what was seen in wild-type mice. Next steps include developing small molecules that modulate LIF receptor signaling.	Not patented; unlicensed	Kubota, Y. *et al. J. Clin. Invest.*; published online June 2, 2008; doi:10.1172/JCI34882 **Contact:** Toshio Suda, Keio University, Tokyo, Japan e-mail: sudato@sc.itc.keio.ac.jp **Contact:** Yoshiaki Kubota, same affiliation as above e-mail: ykubo33@sc.itc.keio.ac.jp

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 40 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
Bifunctional ligands for targeting B cells	Bifunctional ligands, comprised of a ligand of CD22 linked to a nitrophenol (NP) antigen, could be useful for targeting B cells in hematological cancers. The bifunctional ligand was designed to simultaneously bind CD22, found on the surface of B cells, and a monoclonal anti-NP IgM. BJAB K20 human B lymphoma cells incubated with analogs of the bifunctional ligand had more bound anti-NP IgM and greater formation of antibody-CD22 complexes than untreated controls. Next steps include demonstrating that the bifunctional ligand can deliver drug-loaded liposomes to B cells.	Patents pending for bifunctional ligands in drug delivery; available for licensing from The Scripps Research Institute	O'Reilly, M.K. *et al. J. Am. Chem. Soc*; published online May 28, 2008; doi:10.1021/ja802008q **Contact:** James C. Paulson, The Scripps Research Institute, La Jolla, Calif. e-mail: jpaulson@scripps.edu
Cationic liposome carriers for photodynamic therapy	An *in vitro* study suggests that cationic liposomes could be used as carriers of light-activated therapeutics to treat bacterial infections. In a proof-of-concept study, the photosensitizer *m*-tetrahydroxyphenylchlorin was encapsulated in liposomes formulated with cationic surfactants and dimyristoyl-*sn*-glycero-phosphatidylcholine (DMPC). One formulation killed almost all bacteria of a methicillin-resistant *Staphylococcus aureus* strain after five minutes of irradiation, thus showing bactericidal activity comparable to that of free photosensitizer chlorin. Next steps include determining the morphological and physicochemical liposome features that allow for efficient drug delivery.	Not patented; available for licensing through the National Research Council (CNR)	Bombelli, C. *et al. Mol. Pharm.*; published online May 30, 2008; doi:10.1021/mp800037d **Contact:** Giovanna Mancini, CNR, Methodological Chemistry Institute and Sapienza University of Rome, Rome, Italy e-mail: giovanna.mancini@uniroma1.it
Combined analysis of mutational and methylation alterations in cancer candidate (CAN) genes	An *in vitro* study suggests that combined analysis of mutational and methylation alterations could be more informative than mutational analysis alone for identifying genetic risk markers in breast and colon cancer. Breast and colon cancer cells from cell lines or primary tumors had a significantly higher incidence of CAN gene hypermethylation than of somatic gene mutations in the same CAN genes (44% vs. 6%, p=0.004). Hypermethylation of the same genes was observed across multiple lines of breast and colon cancer, whereas mutations were limited to individual lines. Next steps include screening for hypermethylation of the identified genes over a broader range of cancers and identifying additional epigenetically modulated genes associated with cancer risk.	Assays for abnormal DNA methylation patterns are patented; licensed to Oncomethylome Sciences S.A.	Chan, T.A. *et al. PLoS Med.*; published online May 27, 2008; doi:10.1371/journal.pmed.0050114 **Contact:** Stephen B. Baylin, The Johns Hopkins Kimmel Cancer Center, Baltimore, Md. e-mail: sbaylin@jhmi.edu **Contact:** Nita Ahuja, same affiliation as above e-mail: nahuja@jhmi.edu **Contact:** Kornel E. Schuebel, same affiliation as above e-mail: kornels@jhmi.edu
Detection of protein trafficking and turnover in cultured cells or whole animals	A method called time-specific tag for the age measurement of proteins (TimeSTAMP) may be useful for visualizing protein synthesis and trafficking that occurs in the development of tumors, synapses and multiple cell types. The technique uses a covalent epitope tag with self-removing, sequence-specific protease activity. TimeSTAMP detected newly synthesized proteins in cultured cells and growing synapses in cultured neurons, and it visualized recently synthesized proteins in whole fly brains. Next steps include adapting TimeSTAMP to other antibody-based detection methods.	Patent application filed for TimeSTAMP; available for licensing through the University of California, San Diego Technology Transfer Office	O'Reilly, M.K. *et al. Proc. Natl. Acad. Sci. USA*; published online May 26, 2008; doi:10.1073/pnas.0803060105 **Contact:** Roger Y. Tsien, University of California, San Diego, La Jolla, Calif. e-mail: rtsien@ucsd.edu **Contact:** Michael Z. Lin, same affiliation as above e-mail: mlin@ucsd.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
MRI to image pH gradients in diseased tissue *in vivo*	Studies in mice suggest that MRI modified to detect injections of hyperpolarized $H^{13}CO_3^-$ could detect aberrant pH gradients in diseased and cancerous tissue. Tumors show lower pH than normal tissue due to more lactic acid production, lower interstitial fluid buffering and less perfusion. In mice with subcutaneous lymphomas, injection of polarized $H^{13}CO_3^-$ enhanced MRI sensitivity enough to detect $^{13}CO_2$ and $H^{13}CO_3^-$ levels, which are used to calculate pH. Imaging tumor slices excised from the mice gave an average calculated pH of 6.71 compared with imaging tumors within the mice, which gave an average pH of 7.09. Further preclinical studies are necessary to advance the technology into the clinic.	Metabolic MRI technology patented by GE Healthcare; the researchers are interested in partnering to help move the application into the clinic	Gallagher, F. *et al. Nature*; published online May 29, 2008; doi:10.1038/nature07017 **Contact:** Kevin Brindle, University of Cambridge, Cambridge, U.K. e-mail: kmb1001@cam.ac.uk
Solid-state nuclear magnetic resonance (ssNMR) for designing neurodegenerative disease therapies	An *in vitro* study suggests that ssNMR may be useful for developing and identifying compounds to treat neurodegenerative diseases. ssNMR-based analysis of an α-synuclein peptide fragment (71–82) showed that the six C-terminal residues (77–82) play a predominant role in forming insoluble aggregates. α-synuclein is associated with Lewy bodies found in Parkinson's disease (PD) and with amyloid plaques found in Alzheimer's disease (AD). The data on the six residues were used to design a peptide with an *N*-methyl group at the C-terminal residue that inhibited the aggregation of α-synuclein. Next steps include developing modified analogs of the N-methylated peptide with better protease resistance and blood-brain barrier permeability. The ssNMR design procedure could potentially be applied to other proteins and peptides that are associated with amyloid diseases.	Work on N-methylated peptides for amyloid inhibition is patented; licensed to Senexis Ltd.	Madine, M. *et al. J. Am. Chem. Soc*; published online May 30, 2008; doi:10.1021/ja075356q **Contact:** David A. Middleton, University of Liverpool, Liverpool, U.K. e-mail: middleda@liv.ac.uk

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