

THIS WEEK**ANALYSIS****COVER STORY****1 Cancer grows on SOD1**

Researchers from Attenuon have found that SOD1 is a master regulatory switch for kinase phosphorylation involved in angiogenesis and cell proliferation. SOD1 thus is a potential target for a variety of cancers and might have some utility in degenerative diseases as well.

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Researchers from FivePrime have published proof of concept for the company's secreted protein discovery engine, which was used to identify IL-34. The previously unknown protein is involved in monocyte proliferation, but its functional role in a host of potential diseases now needs to be elucidated.

6 Moving upstream of diabetes

With no approved treatments for metabolic syndrome, two papers in *PNAS* point to a trio of targets for treating two of the syndrome's key features—obesity and insulin resistance. More animal work will determine the best choice of the three targets: a TGF- β receptor called ALK-7 or either of its two ligands, activin B and GDF-3.

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Although chemo- and radiotherapy directly attack gliomas, they do not address the neurodegeneration and edema that result from the tumor's invasion. A paper in *Nature Medicine* suggests both effects can be addressed by hitting the same target—the glutamate transporter xCT.

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By Michael J. Haas, Senior Writer

A report in the *Proceedings of the National Academy of Sciences* shows how superoxide dismutase 1 acts as a master regulatory switch for kinase phosphorylation in angiogenesis and cell proliferation, making it a potential target to treat a variety of cancers.¹ Although some mechanistic details remain to be elucidated, superoxide dismutase 1 inhibition could be used in combination with other cancer treatments and might have utility in some degenerative diseases as well.

Superoxide dismutase 1 (SOD1) is one of three SOD enzymes that catalyze the conversion of intracellular superoxide to hydrogen peroxide. Intracellular superoxide is produced when a growth factor binds to its receptor on the cell surface and induces receptor phosphorylation (see **Figure 1, "Superoxide dismutase 1 in cancer"**).

Recently, SOD1 was identified as the target of tetrathiomolybdate (TTM),² a compound that has antiangiogenic and antitumor activity in mice,³⁻⁵ but whose mechanism of action remained unclear.

In the *PNAS* paper, short interfering RNA and ATN-224, a second-generation TTM from **Attenuon LLC**, were used to study SOD1 inhibition in human umbilical vein endothelial cells (HUVECs) and multiple myeloma (MM) tumor cells.

The research team was led by Fernando Doñate while he was associate director of biology at Attenuon and included scientists from **Cold Spring Harbor Laboratory** and **D.E. Shaw Research**. Doñate is presently director of preclinical R&D at **Proacta Inc.**

The researchers found that in proliferating cells, hydrogen peroxide oxidizes protein tyrosine phosphatases (PTPs)—such as PTP-1B—thus blocking their dephosphorylation activity. PTP inactivation in turn upregulates the kinase cascade, which ultimately drives phosphorylation of MAP kinase ERK-1 and MAP kinase ERK-2, both involved in angiogenesis and cell growth.

When SOD1 was inhibited, hydrogen peroxide levels decreased and PTPs remained active, resulting in reduced phosphorylation of ERK-1 and ERK-2.

"The kinases are still working, but the phosphatases win the battle," Doñate said. "This results in attenuation—not complete shutdown—of the phosphorylating kinase pathways."

Complete inhibition of SOD1 with ATN-224 induced apoptosis in most cell lines the team tested, but Doñate said they have not yet determined how this occurred.

Taken together, he said the results indicate ATN-224 could be complementary to tyrosine kinase inhibitor therapy for cancer. Such



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a strategy would both “hit the kinases and activate the phosphatases in tumors and endothelial cells,” he said.

Previous studies by research teams at **Wayne State University** and **Stanford University** showed that SOD1 knockout mice are generally healthy, though they are more prone to liver cancer and some female mice have decreased fertility.^{6,7}

“The fact that SOD1 knockout mice have a mild phenotype supports the theory that resting cells can tolerate SOD1 being inhibited,” making SOD1 a potentially attractive target in cancer cells, Doñate told *SciBX*.

Andrew Mazar, CSO at Attenuon and coauthor of the *PNAS* paper, told *SciBX* that ATN-224 is in four clinical trials: a Phase I/II trial in MM; Phase II trials for prostate cancer and metastatic melanoma, with data for both expected this year; and a Phase II trial in breast cancer, which began in April.

New ground for SOD1

Company representatives contacted by *SciBX* agreed that targeting levels of reactive oxygen species (ROS) like superoxide is a viable strategy for cancer, but differed in their opinions on whether SOD1 inhibition was the best way to do so.

“The *PNAS* paper shows an interesting new function for SOD1 that I don’t think was appreciated until this was published,” said Frank Bennett, SVP of research at **Isis Pharmaceuticals Inc.**

“It is becoming widely acknowledged that levels of ROS are elevated in a variety of acute and chronic inflammatory conditions including cancer, diabetes, neurodegenerative, renal and cardiovascular diseases,” said Chris Wigley, VP of research at **Reata Pharmaceuticals Inc.**

Thus, Wigley said, it is encouraging to see work on therapeutic strategies that aim to influence ROS levels to modulate signaling pathways. “We anticipate the commercial development of such therapeutics will have dramatic implications for a vast array of inflammatory conditions,” he said.

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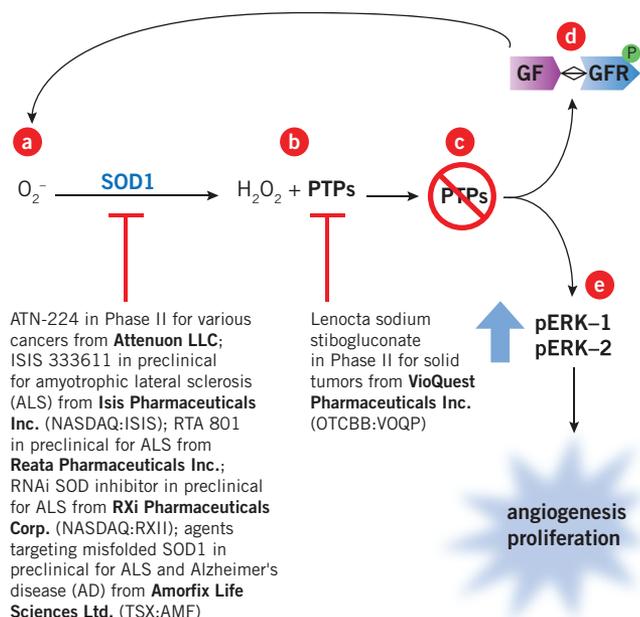


Figure 1. Superoxide dismutase 1 in cancer. The elucidation of the role of superoxide dismutase 1 (SOD1) in angiogenesis and proliferation opens the door to new therapeutic approaches in cancer. Binding of growth factors (GFs; for example, epidermal growth factor, insulin-like growth factor-1 and fibroblast growth factor-2) to their respective receptors (GFRs) induces receptor phosphorylation, which produces superoxide (O_2^-) by mechanisms not firmly established [a]. In the cytoplasm, superoxide is converted to hydrogen peroxide (H_2O_2) through the superoxide dismutation reaction carried out by the catalyst SOD1 [b], and H_2O_2 then oxidizes protein tyrosine phosphatases (PTPs) such as PTP-1B. In turn, the dephosphorylation activity of PTPs is decreased [c], allowing the kinase cascade to proceed. This results in a feedback cycle that drives ongoing phosphorylation of GFRs [d] and upregulation of phosphorylated MAP kinase ERK-1 and phosphorylated MAP kinase ERK-2 (pERK-1; pERK-2) [e], leading to angiogenesis and cell proliferation.

However, Wigley also suggested that targeting SOD1 to affect ROS levels might be too narrow a strategy given cancer's propensity to develop resistance to single-target therapies.

"A more advantageous approach to modulating ROS levels would be to increase cellular antioxidant capacity by inducing the production of a host of antioxidant enzymes and molecules," he said. "Such a general antioxidant approach to inhibiting ROS-mediated growth signaling is desirable, as opposed to selectively targeting a single enzyme," like SOD1, which decreases peroxide but increases superoxide.

Wigley added that a more general approach also would be desirable because of the tendency of SOD1 knockout mice to develop hepatic malignancies.

Reata's RTA 402 takes just such a general approach. The compound is a synthetic triterpenoid that induces the anti-inflammatory transcription factor NF-E2-related factor 2 (Nrf2) and inhibits the transcriptional activities of NF- κ B and signal transducer and activator of transcription 3 (STAT3).

RTA 402 is in two Phase I trials to treat advanced solid tumors or lymphoid malignancies. It is also in a Phase I/II trial to treat pancreatic cancer, with completion expected this year.

In April, Reata began a Phase II trial of the compound to treat diabetic nephropathy, and Wigley said the company plans to begin Phase II trials in additional inflammatory indications in 2H08.

Vernon Alvarez, VP of R&D at **VioQuest Pharmaceuticals Inc.**, said the results reported in *PNAS* show that SOD1 plays an important role in cancer. "But it is also evident that it needs to be completely abrogated in order to have full antiproliferative effects," he said.

VioQuest is developing Lenocta sodium stibogluconate, an inhibitor of Src homology PTPase-1 (SHP-1), SHP-2 and PTP-1B. It is in a Phase IIa trial in combination with interferon- α to treat advanced solid tumors, primarily melanoma and renal cell carcinoma (RCC).

Because Lenocta inactivates PTP-1B differently than ATN-224 does, "Lenocta may prove to be synergistic with SOD1 inhibition," Alvarez said. "Such a combination would be very interesting to study," he sug-

gested, first for its effects on the expression of active PTP-1B and then *in vitro* and *in vivo* to determine whether the effect was synergistic.

"The role that PTPs play in angiogenesis is just beginning to be appreciated, but it is clear that many of the same PTPs are activated in angiogenesis" across tumor types, he said. "The impact of these PTPs to cancer cannot be overstated, but there is clearly much research to be done."

ALSo applicable?

SOD1 also has been implicated in amyotrophic lateral sclerosis (ALS).⁸

Isis's Bennett said ATN-224 is unlikely to provide any benefit in familial or inherited ALS, which comprises about 20% of all cases. A fraction of these familial cases are the result of a genetic mutation that causes SOD1 to fold abnormally, "without affecting its enzyme activity," he said. Instead, the misfolded SOD1 has abnormal protein-protein interactions, resulting in aggregates that characterize the disease.

Bennett said inhibiting SOD1 activity with a compound like ATN-224 is probably not useful for ALS because it does not remove the protein from the brain and therefore would not be expected to prevent the misfolded SOD1 from forming aggregates. "Instead, Isis aims to inhibit the expression of the SOD1 protein," he said.

But Bennett added that the *PNAS* paper did raise some questions worth investigating, such as whether the misfolded SOD1 can still bind ATN-224 and whether the compound's binding of misfolded SOD1 would prevent aggregation.

He said the latter is unlikely, but noted, "It's a prediction—and predictions can be wrong—so it's worth testing."

The company has ISIS 333611, an antisense oligonucleotide targeting SOD1, in preclinical toxicity studies. Isis plans to start a Phase I trial in familial ALS this year.

Reata's Wigley also said SOD1 inhibition would not necessarily be a benefit in ALS, noting that ATN-224 binds SOD1's copper ion, which is required for proper SOD1 structure and activity.

He said SOD1 without copper has been shown to be less structurally

stable than it is with copper, and the structural instability of misfolded SOD1 in familial ALS correlates with disease severity.

Reata's RTA 801, a stabilizer that targets misfolded SOD1, is in pre-clinical development to treat ALS. Wigley said the company is working to identify additional SOD1 modulators for the disease.

Doñate agreed with both Bennett and Wigley that ATN-224 would probably have little use in ALS. "But ATN-224 could be useful in other degenerative diseases where copper is involved, such as Alzheimer's and Wilson's diseases," he said.

Over the last decade, a growing body of evidence has implicated copper ions in the formation of the amyloid plaques that characterize Alzheimer's disease (AD), though the exact nature of that role is not fully understood.⁹⁻¹¹

Until early this year, **Pipex Pharmaceuticals Inc.** was developing Coprexa, a first-generation oral TTM to treat Wilson's disease, an autosomal recessive genetic disorder characterized by the accumulation of copper in the brain, liver and other tissues. In January, however, the company received a refusal-to-file letter from the FDA for an NDA for the compound.

Unanswered questions

The *PNAS* paper raises several follow-on questions relevant to cancer research, according to Doñate.

One is whether SOD1-mediated inhibition of growth factor receptor phosphorylation is sufficient to cause apoptosis. Doñate said, "We think not—that maybe something else is going on," but declined to elaborate.

Another question is what effect higher levels of superoxide—a consequence of SOD1 inhibition—have on the cell. He said the team presented data in the *PNAS* paper indicating some level of oxidative damage to the cell but added that "SOD1 knockdown suggests a tolerance for superoxide in nonproliferating cells."

He said the team had not investigated the contribution of higher superoxide levels to apoptosis in ATN-224-treated cancer cells.

A final question is how SOD1 inhibition downregulates expression of platelet-derived growth factor- β receptor as reported in the paper.

Doñate said the team has no immediate plans to continue investigating the SOD1 mechanism in cancer, but has collaborators with whom it might work on SOD1 in the future.

Proacta, Doñate's current company, is developing hypoxia-activated prodrugs for use in combination with other chemotherapies. Attenuon, his former company, has filed a patent on the mechanism of SOD1 in cancer.

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FivePrime's time

By Lauren Martz, Staff Writer

A paper published in *Science* has identified the previously unknown protein IL-34 as being involved in monocyte proliferation.¹ Besides opening new doors for autoimmune disease and cancer research, the finding provides proof of concept for the secreted protein discovery platform of **Five Prime Therapeutics Inc.**, a company that has been running quietly since raising \$45 million to fund preclinical research in 2005.

FivePrime was founded with the goal of industrializing secreted protein discovery and development. Its technological platform includes a library of secreted proteins and extracellular domains of transmembrane proteins, and assays that measure the metabolic, growth and transcriptional responses of each protein. Analysis of the assay data allows the company to select proteins with specificity for desired assays and thus for desired properties.

In the *Science* paper, Lewis Williams and colleagues put this discovery engine to work to identify a protein that regulates monocytes with the hope of finding a protein that could be targeted to treat cancer or autoimmune disease. Monocytes mature to form macrophages and are essential for the immune system's recognition and destruction of foreign bodies. Monocytes are thus a line of defense against tumor cells, whereas improper monocyte activation can cause autoimmune diseases.²

Williams, the corresponding author on the paper, is cofounder and chairman of FivePrime. He is also an adjunct professor at the Cardiovascular Research Institute of the **University of California, San Francisco**.

The team scanned about 3,400 secreted proteins and extracellular domains in 25 assays and selected IL-34 for further research after functional studies showed that purified IL-34 promoted monocyte viability. An assay measuring DNA replication showed the enhanced viability was due to cell proliferation rather than just increased survival. In human bone marrow cultures, IL-34 promoted formation of a macrophage precursor, which further supports the cytokine's role in monocyte maturation.

Researchers also found that this previously unknown growth factor is expressed in a variety of cell types including cardiac, brain, lung, liver, kidney, spleen, thymus, testes, ovary, small intestine, prostate and colon.

"Because we are dealing with a ligand expressed in such a range of cell types that acts on a receptor with very broad potential clinical applications, the most critical next step in pursuing IL-34 as a therapeutic target is to decide the best indication. We are in the midst of animal studies for IL-34 antagonists," Williams told *SciBX*.

The target

Although IL-34 is a newly discovered protein, additional results reported in the *Science* paper reveal that its receptor is not. Through a subsequent screening of 858 extracellular protein domains, the

researchers identified IL-34's receptor as the previously described and well-studied colony-stimulating factor-1 receptor (CSF-1R).

Until now, CSF-1, the primary regulator of the development, survival, proliferation and differentiation of macrophages, was the only known ligand for CSF-1R.³ The new findings raise the question of whether targeting IL-34 would have benefits over targeting CSF-1.

Richard Stanley, chairman of the Department of Developmental and Molecular Biology at the **Albert Einstein College of Medicine**, told *SciBX* the CSF-1 ligand/receptor pair has been implicated in the regulation of innate immunity, and activation of the receptor might be associated with development of atherosclerosis, osteoporosis, kidney allograft rejection, collagen-induced arthritis, leukemia and obesity. Thus, he said, antagonists of the receptor could have broad therapeutic potential.

Steven Bass, senior director of R&D at **Maxygen Inc.**, told *SciBX* that a new ligand for CSF-1R is a welcome addition to the pool of potential therapeutics. "CSF-1R is a proto-oncogene. As such, you might want to block IL-34" to treat cancer, he said.

Maxygen's MAXY-G34 is a next-generation G-CSF that is in Phase II testing to treat chemotherapy-induced neutropenia in breast cancer patients.

Heishan Lin, first author on the paper and director of immunology at FivePrime, agreed that cancer is indeed an area where IL-34's regulation of macrophage colony-forming cell development could have therapeutic potential. Lin also noted that IL-34 "can give rise to osteoclasts, which are relevant to osteoporosis, and microglia, which are implicated in neurological disorders including multiple sclerosis."

Stanley cautioned that although both ligands, IL-34 and CSF-1, act through the same receptor, much more needs to be known about IL-34 before it is considered a valid therapeutic target. "We know IL-34 acts on the CSF-1 receptor. Now what we really need to know is: does it only act on that receptor, and are there still other ligands acting on that receptor as well," he said.

Previous studies in mice showed that knockout of CSF-1 did not cause phenotypic effects as severe as those caused by knockout of its receptor.⁴ With IL-34 in hand, Stanley suggested that "the critical next step is to test whether a double knockout of CSF-1 and IL-34 has the same phenotypic effects as the CSF-1R knockout model. If this is the case, you confirm that these are the two primary ligands acting on the receptor and help rule out the possibility that IL-34 is acting on other receptors, which could cause side effects."

He added, "We understand how CSF-1 and IL-34 are the same; now what we need to understand is how the two differ. We want to find out where their expression patterns do not overlap and how they are differentially regulated."

This is important because "if there is a big overlap, there may be little advantage to targeting IL-34, but if it has selective effects, which are very likely, it could be useful," said Stanley. For example, if inhibition of CSF-1R has side effects, it could be therapeutically advantageous to target its ligands individually.

(Continues on p. 6)

"We understand how CSF-1 and IL-34 are the same; now what we need to understand is how the two differ."

**—Richard Stanley,
Albert Einstein College
of Medicine**

Moving upstream of diabetes

By Lev Osherovich, Senior Writer

There is a growing consensus in the medical community that early intervention is essential for treating diabetes.¹ However, there are no approved treatments for metabolic syndrome, a complex set of conditions that often precedes diabetes. Now, two papers^{2,3} in the *Proceedings of the National Academy of Sciences* point to a trio of targets for treating obesity and insulin resistance, two key features of metabolic syndrome.

The studies suggest that targeting growth differentiation factor-3 (GDF-3) could reduce obesity, hitting activin B could boost insulin secretion and inhibiting the transforming growth factor- β receptor (TGF- β receptor) activin receptor-like kinase-7 (ALK-7) could do both—GDF-3 and activin B are both ALK-7 ligands.

Industry and academic researchers told *SciBX* that targeting this group of proteins is a new angle of attack for metabolic syndrome, but they added that the question of which of the targets to hit will require further preclinical study.

Acceleron Pharma Inc. has licensed the discoveries and already has ALK-7 derivatives in preclinical development.

The papers are both from a group led by Carlos Ibañez, professor of neuroscience at the **Karolinska Institute**.

“This is the first clear-cut demonstration of the normal physiological role of GDF-3 in regulating metabolism.”

—Se-Jin Lee,
Johns Hopkins University

In one study, Ibañez’s team examined how knocking out ALK-7 and its ligands affected insulin secretion in the pancreas. “We have found a new mechanism to control insulin release by β -cells,” Ibañez told *SciBX*. The ALK-7 circuit acts “to control the amount of insulin released in response to glucose,” he added.

The second study focused on the metabolic functions of GDF-3, an alternative ALK-7 ligand that is secreted by adipocytes. Here, Ibañez’s team discovered a separate role for ALK-7 in regulating obesity.

“This is the first clear-cut demonstration of the normal physiological role of GDF-3 in regulating metabolism,” said Se-Jin Lee, professor of molecular biology and genetics at **Johns Hopkins University**.

Fat liver, skinny mouse

ALK-7 is a membrane-bound receptor for several TGF- β homologs that are involved in developmental patterning and cell growth.⁴ Ibañez said his team found that despite developing normally, ALK-7 knockouts had higher serum insulin levels than wild-type controls. The mutant mice also developed enlarged β -islet cells and fatty livers.

Ibañez told *SciBX* that knocking out ALK-7 created a distinct form of insulin misregulation. “These mice are not diabetic, in that they still have normal glucose” when they are young, he said. “Young mice have high insulin in the blood, but are not insulin resistant.”

However, the mice developed insulin resistance as they got older. Ibañez thinks this resulted from compensation in glucose sensing after a lifetime of high insulin.

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(Continued from "FivePrime's time," p. 5)

Whereto next?

Whatever the final fate of IL-34 as a drug target, the *Science* paper has demonstrated that FivePrime’s comprehensive discovery platform will most likely be useful for identifying additional therapeutic targets. “Being able to find a novel cytokine that has been hiding under our noses is impressive,” said Bassil Dahiyat, CEO of **Xencor Inc.**

Dahiyat added that an advantage of the platform is its use of cell-based assays rather than binding or panning assays, “which give much less information and are so much more difficult to deduce relevant biology from.” Dahiyat and Stanley both told *SciBX* that increasing the number of functional assays would further increase the success of protein discovery platforms such as FivePrime’s.

Xencor’s Protein Design Automation (PDA) technology is used to optimize antibodies for cancer and therapeutic proteins for inflammation and autoimmune disorders. The company’s XmAb 2513, a humanized monoclonal antibody against CD30, is in Phase I testing to treat cancer.

In addition to the IL-34 paper in *Science*, FivePrime’s discovery engine received a further validation boost because of last week’s deal with **Pfizer Inc.** to discover antibody targets and protein therapeutics to treat cancer and diabetes. The biotech said the deal does not include IL-34.

Since 2005, Williams said, “we’ve moved a compound near IND filing, have three additional preclinical development candidates, a number of other potential lead molecules, a new *in vivo* screening platform and have expanded the company to about 85 individuals.”

He told *SciBX* that lead compound FP-1039, an extracellular domain molecule to treat cancer, is within a few weeks of an IND submission.

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COMPANIES AND INSTITUTIONS MENTIONED

- Albert Einstein College of Medicine**, Bronx, N.Y.
- Five Prime Therapeutics Inc.**, San Francisco, Calif.
- Maxygen Inc.** (NASDAQ:MAXY), Redwood City, Calif.
- Pfizer Inc.** (NYSE:PFE), New York, N.Y.
- University of California, San Francisco**, Calif.
- Xencor Inc.**, Monrovia, Calif.

To determine how ALK-7 regulates insulin production, the researchers tested the effect of various ALK-7 ligands on insulin secretion in isolated pancreatic β -islet cells. The team found that knocking out activin B recapitulated the increased insulin secretion found in ALK-7 knockouts, thus suggesting that activin B inhibits insulin production by binding to ALK-7.

The second paper shows that ALK-7 wears a second hat as a regulator of fat deposition. When fed a high-fat diet, ALK-7 knockouts did not gain as much weight as wild-type controls did despite comparable food intake.

To uncover how ALK-7 controlled fat, Ibañez turned to GDF-3, which previous studies had implicated in fat accumulation in adipocytes.⁵ Just like ALK-7 knockouts, GDF-3 knockouts proved resistant to weight gain induced by a high-fat diet compared with wild-type controls, suggesting that the receptor and ligand work together in adipose tissue.

Consistent with this idea, Ibañez's team found that ALK-7 was expressed in adipocytes and could be activated by GDF-3.

Together the studies show that activin B and GDF-3 control distinct functions of ALK-7. Indeed, overfed ALK-7 knockouts were lean but had high insulin levels, whereas overfed GDF-3 knockouts were lean despite normal insulin levels. Ibañez told *SciBX* that ongoing experiments are testing whether activin B knockouts, which have high insulin levels, are fat or lean when on a high-fat diet.

Angles of approach

Several therapeutic approaches for treating metabolic syndrome or diabetes could emerge from the *PNAS* papers. However, questions remain about which of the targets to hit and whether it is better to increase insulin secretion, reduce fat deposition or do both.

According to Jasbir Sehra, CSO of Acceleron, the ALK-7 system presents multiple targets, each with its own advantages and drawbacks.

At first blush, the clear target would be ALK-7 because blocking the receptor both reduces obesity and increases insulin. However, Sehra cautioned that ALK-7 may have other ligands with important functions, and thus knocking down the receptor may have side effects.

For treating obesity alone, Sehra thinks that inhibiting "ALK-7 will be powerful but not so selective," whereas inhibiting GDF-3 "may provide the desired effect."

Ibañez agreed and told *SciBX* that for obesity, "GDF-3 would be a good target, but we don't know as much about it" as other TGF- β proteins.

Targeting activin B could control glucose levels in patients with metabolic syndrome, and this approach is under consideration at Acceleron, according to Sehra.

Acceleron makes soluble fragments of TGF- β receptors to treat a range of diseases. The company's lead compound, ACE-011, a fusion protein combining the activin-binding portion of activin receptor type 2a with soluble IgG, is in Phase Ib trials for osteoporosis. The compound is partnered with **Celgene Corp.**

Sehra told *SciBX* that Acceleron is developing a soluble fragment of ALK-7 called ACE-06X and is optimizing the compound's binding affinity for GDF-3 using protein engineering.

In support of this approach, Ibañez pointed out that unlike ALK-7 knockout mice, the activin B knockouts produced excess insulin but did not develop insulin resistance late in life.

It is possible that the ALK-7 system's true calling is in treating full-blown type 2 diabetes. At least six approved diabetes drugs control glucose levels by stimulating insulin secretion in β -cells.⁶

Francine Gregoire, associate director of *in vivo* pharmacology at **Metabolex Inc.**, told *SciBX* that inhibiting the activin B branch of ALK-7 activity could be a good approach to "improvement of islet health and enhancement of β -cell mass."

In March, Metabolex started a Phase I trial of MBX-2982, an agonist of a β -cell receptor called G protein-coupled receptor 119 (GPR119), to treat type 2 diabetes. Activation of GPR119 stimulates the secretion of insulin by β -cells.⁷

At the end of the day, both Sehra and Ibañez agree that the specific approach will depend on the indication.

"It may be that you have to inhibit the receptor in certain diseases and the ligands in others," said Sehra.

Next steps

The biggest question is whether targeting the ALK-7 system can affect insulin levels or obesity in genetically normal adult animals. TGF- β proteins are best known as embryonic morphogens, and some researchers cautioned that some of the observed phenotypes in the two studies may result from developmental effects.

According to Sehra and Lee, the proof of therapeutic utility will be to administer antibodies against the three targets to adult mice and then monitor insulin and fat levels.

Moreover, the specific tissues in which these proteins act are not yet certain.

"We don't know which site of expression is responsible for the effects" of ALK-7, Ibañez said. "It's placed in a lot of tissues that could have an impact."

Ibañez plans to develop β -islet cell-specific knockouts to test whether ALK-7, activin B and GDF-3 act locally. An alternative mechanism might be that these proteins affect metabolism through the nervous system, like the appetite-regulating hormones leptin and ghrelin.

Gregoire agreed with Ibañez's plan, noting that potential "metabolic perturbations in other tissues" resulting from ALK-7 inactivation need to be explored "with tissue-selective knockout models."

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Swell effect on glioma

By Michael J. Haas, Senior Writer

Gliomas, unlike most other tumor types, grow and expand by killing off surrounding tissue, causing neurodegeneration and edema. Chemotherapy and radiotherapy attack the tumor itself, but do not address these two other effects of the cancer. A paper in *Nature Medicine* suggests that neurodegeneration and edema can be alleviated by hitting one target—the glutamate transporter xCT—making selective inhibition of xCT a potentially useful adjunct to glioma treatments.

For a nearly a decade it has been known that the neurodegeneration associated with gliomas results from their release of excess glutamate triggered by xCT overexpression.^{1,2} The *Nature Medicine* paper now reports that the induced neurodegeneration is a significant—if not the exclusive—contributor to brain edema.

The study, from researchers at the **Swiss Federal Institute of Technology Zurich**, the **University of Zurich**, the **Netherlands Cancer Institute**, the **University of Erlangen-Nuremberg (FAU)** and the **University of Cologne**, showed that selective inhibition of xCT reduced neurodegeneration and edema and that it conferred a survival benefit despite having no effect on tumor proliferation.³

The research team was led by Ilker Eyüpoğlu, neurosurgeon and principal investigator at FAU. According to Eyüpoğlu, the prevailing theory has been that glioma-induced brain edema results from leaky tumor blood vessels and disruption of the blood-brain barrier during tumor angiogenesis. Both processes allow entry of fluids that do not ordinarily cross into the brain.

“In our work we provide evidence that glutamate secretion and cytotoxic events—neuronal cell death—contribute to the edema formation,” he told *SciBX*.

xCT role

xCT is a transmembrane protein expressed on neurons, astrocytes and gliomal cells. It mediates cellular secretion of glutamate in exchange for cystine. Cystine is required for the synthesis of the antioxidant glutathione (GSH), which protects cells from free radicals and other oxidizing toxins.

The role of xCT in glioma-induced neurodegeneration was first elucidated in 1999 by researchers from the **University of Alabama at Birmingham (UAB)** and **Johns Hopkins University**, led by Harald Sontheimer, professor of neurobiology at UAB.^{1,2} Those studies showed that gliomas overexpress xCT and release excessive amounts of extracellular glutamate, killing the surrounding neural tissue through excitotoxicity. They also showed that overexpression of xCT increased gliomal uptake of cystine, resulting in higher GSH levels that increased the tumor’s resistance to radio- and chemotherapies.

Sontheimer and colleagues identified the xCT inhibitor,

S-4-carboxyphenylglycine (S-4-CPG), used in the recent *Nature Medicine* paper.

According to Eyüpoğlu, those earlier studies with S-4-CPG focused on how reducing GSH within the glioma affected tumor size, and they used high concentrations of the compound (250–500 µM).

“In our paper, we provide data that S-4-CPG is already potent enough to inhibit glutamate secretion below 100 µM without affecting proliferation,” he said, which allowed the team to study the effects of inhibiting glutamate secretion in the absence of changes in tumor size.

To investigate those effects, the team used short interfering RNA and S-4-CPG to confirm that blocking xCT in glioma cells and human brain tissue samples reduced glutamate secretion and subsequent neuronal damage.

“How or whether neurodegeneration and edema contribute to mortality has been hard to tease apart from the tumor’s contribution. But these results imply that neurodegeneration leads to lower survival rates.”

—Andrew Mazar, *Attenuon LLC*

Next, the team observed that rats injected with xCT-silenced gliomas had delayed neurodegeneration and prolonged survival compared to control rats injected with untreated gliomas.

Then the team showed that low doses of S-4-CPG delayed onset of neurodegeneration, reduced edema and prolonged survival in control rats, even though tumor volume remained unchanged.

Eyüpoğlu noted that previous studies demonstrated xCT is “dispensable for normal development, cell proliferation and health” in mammals.^{4,5} Thus, he said, selective targeting of

xCT could treat both edema and neurodegeneration in glioma patients without significant side effects.

UAB’s Sontheimer told *SciBX* that his own research teams have not studied glioma-induced edema, “so this aspect of the *Nature Medicine* paper may well be new.” He did note that edema can also result from tumor necrosis.

Eyüpoğlu said his team is exploring the extent to which glioma-induced neurodegeneration contributes to brain edema, relative to other possible factors such as tumor growth and necrosis.

Adjuvant therapy

Companies told *SciBX* that xCT inhibitors might work as adjuncts to existing chemotherapies, but they expect that S-4-CPG may not be the optimal candidate.

Andrew Mazar, CSO of **Attenuon LLC**, said that “to see something that reduces both glioma-related neurodegeneration and edema *in vivo* is a significant advance.” He said that the 30% increase in survival observed with low doses of S-4-CPG is also noteworthy—especially in the absence of any effect on tumor size.

“How or whether neurodegeneration and edema contribute to mortality has been hard to tease apart from the tumor’s contribution,” Mazar said. “But these results imply that neurodegeneration leads to lower survival rates.”

Thus, he said, an xCT inhibitor could be useful in combination with a drug that both increases survival and shrinks tumors, such as Avastin bevacizumab, an antibody to VEGF from **Genentech Inc.** and **Roche** that is marketed for colorectal cancer and non-small cell lung cancer (NSCLC).

Glioma is typically treated with surgery followed by chemotherapy. However, companies are pursuing the development of combination therapies. For example, the combination of Avastin and Camptosar irinotecan, a topoisomerase inhibitor from **Pfizer Inc.**, is in a Phase III trial to treat recurrent glioblastoma multiforme.⁶

Mazar thinks that “Avastin plus irinotecan is on the way to becoming the new standard of care.”

Attenuon is developing ATN-161, a five-residue peptide derived from fibronectin that targets integrin $\alpha_5\beta_1$ and integrin $\alpha_v\beta_3$. Last year, the company halted a Phase II trial of an i.v. formulation of the compound in intracranial malignant glioma to reformulate it for subcutaneous injection. Attenuon expects to reenter the clinic within 18 months.

Alistair Stewart, director of corporate development at **Allon Therapeutics Inc.**, said the inability of S-4-CPG to cross the blood-brain barrier presented a significant hurdle—but not an insurmountable obstacle—to its use in treating neurodegenerative indications.

“Small lipophilic molecules will cross the blood-brain barrier,” but molecules that are too large or too polar, such as S-4-CPG, will be excluded from the brain, he said.

Allon’s AL-309, a nine-amino-acid peptide, is in preclinical studies as a neuroprotectant to treat Alzheimer’s disease and amyotrophic lateral sclerosis (ALS).

Stewart suggested that one way around this barrier might be to deliver S-4-CPG during surgery, in a manner similar to Gliadel Wafer from MGI Pharma Inc., a subsidiary of **Eisai Co. Ltd.** This carmustine implant is a chemotherapeutic agent implanted in the brain upon the surgical removal of gliomas.

Another potential solution is to use a different xCT inhibitor, such as sulfasalazine, a generic that is marketed to treat Crohn’s disease. In 2005, Sontheimer and colleagues identified this molecule as an xCT inhibitor,⁷ and he and UAB are planning a Phase I trial of the compound as an adjuvant to treat brain cancer.

“If the UAB group has some positive data in their clinical study, someone will pick up on this and try to reposition sulfasalazine,” said Mazar.

“I would be looking to demonstrate the importance of xCT in other conditions, in order to maximize the potential of new drugs against this target.”

**—Alistair Stewart,
Allon Therapeutics Inc.**

Beyond glioma

Allon’s Stewart said he also wants to see whether targeting xCT “has an impact on neuronal survival and edema in other indications.” If so, he said, the *Nature Medicine* findings could have implications for other neurodegenerative indications such as Alzheimer’s disease (AD), stroke and traumatic brain injury.

“This broader application may be needed because glioblastoma is a small market relative to other cancers,” he said. “I would be looking to demonstrate the importance of xCT in other

conditions, in order to maximize the potential of new drugs against this target.”

Meanwhile, the next steps for Eyüpoğlu’s team include studying whether lower levels of cystine and GSH in xCT-silenced tumors indeed make brain tumors more susceptible to chemotherapy. Eyüpoğlu said the findings reported in *Nature Medicine* are not patented.

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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
Cancer				
Cancer	β -tubulin	An SAR study suggests that a new generation of taxanes could be useful for treating paclitaxel-resistant cancers. <i>In vitro</i> , β -tubulin-targeting taxane compounds with modifications at the C2, C10 and C3'N positions showed cytotoxicity against paclitaxel-resistant ovarian cancer cell lines. In murine tumor xenograft models, one of the compounds was active against drug-resistant human ductal pancreatic adenocarcinoma and human colon adenocarcinoma. Further testing in animals is necessary to confirm the safety and efficacy of the modified compounds. The taxane paclitaxel is a generic chemotherapeutic marketed to treat multiple types of cancer. Taxotere docetaxel, also a taxane, is marketed by sanofi-aventis Group to treat multiple cancer indications. No fewer than 15 other companies have paclitaxel-based cancer therapeutics in development.	Compounds patented by the Research Foundation of New York for composition and pharmaceutical application; licensed by sanofi-aventis Group; available for licensing	Ojima, I. <i>et al. J. Med. Chem.</i> ; published online May 9, 2008; doi:10.1021/jm800086e Contact: Iwao Ojima, State University of New York at Stony Brook, Stony Brook, N.Y. e-mail: iojima@notes.cc.sunysb.edu
Cancer	p53; MDM2	Studies in cell culture suggest that constitutive acetylation of the tumor suppressor p53 is important for enabling the p53-mediated stress response and could be useful to treat cancer. <i>In vitro</i> , acetylation of p53 prevented MDM2 from blocking p53 activation. The acetylation inhibited the recruitment of MDM2 to p53-responsive promoters. Loss of acetylation completely blocked p53-dependent growth arrest and apoptosis. Next steps include preclinical testing of known deacetylase inhibitors to evaluate their potential as cancer therapeutics. Ascenta Therapeutics Inc. has three MDM2 inhibitors in preclinical testing for various cancer indications. Nutlin, a small molecule MDM2 antagonist from Roche, is also in preclinical testing to treat cancer.	Not patented; unlicensed	Tang, Y. <i>et al. Cell</i> ; published online May 15, 2008; doi:10.1016/j.cell.2008.03.025 Contact: Wei Gu, Columbia University, New York, N.Y. e-mail: wg8@columbia.edu
Cancer	Signal transducer and activator of transcription 5 (STAT5); IL-3	Studies in cell culture and mice suggest that antagonizing IL-3 could help treat some cancers. In mice implanted with human tumor endothelial cells, IL-3-cultured CD45 ⁺ angiogenic cells were recruited by tumor neovasculature. Also in mice, STAT5 knockout prevented IL-3-dependent neovessel formation, suggesting that STAT5 signaling is necessary for IL-3 activity. Next steps include developing synthetic peptides that interfere with IL-3.	Patented by the University of Torino; unlicensed	Zeoli, A. <i>et al. Blood</i> ; published online May 6, 2008; doi:10.1182/blood-2007-12-128215 Contact: Maria Felice Brizzi, University of Torino, Torino, Italy e-mail: mariafelice.brizzi@unito.it
Cancer	Superoxide dismutase 1 (SOD1); MAP kinase ERK-1; MAP kinase ERK-2	Studies in cell culture suggest that antagonizing SOD1 could be useful for inhibiting angiogenesis and thus treating cancer. Inhibiting SOD1 with short interfering RNA or tetrathiomolybdate (ATN-224) inhibited downstream ERK phosphorylation, which normally contributes to angiogenesis and cell proliferation. Complete inhibition of SOD1 with ATN-224 induced apoptosis in cancer cell lines. Further work is needed to determine how ATN-224 induces apoptosis. ATN-224, a SOD1 inhibitor from Attenuon LLC, is in a Phase I/II trial to treat multiple myeloma (MM) and Phase II trials to treat prostate cancer, breast cancer and metastatic melanoma. Isis Pharmaceuticals Inc. and Reata Pharmaceuticals Inc. each have a SOD1 inhibitor in preclinical development for ALS. (See Cancer grows on SOD1 , page 1.)	Patent application filed for the SOD1 mechanism; patent on ATN-224 already held by Attenuon	Juarez, J. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online May 12, 2008; doi:10.1073/pnas.0709451105 Contact: Fernando Doñate, Proacta Inc., San Diego, Calif. e-mail: fdonate@proacta.com

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Chronic lymphocytic leukemia (CLL)	<i>MDM2</i> (SNP309)	A genotyping study suggests that a SNP in the promoter region of <i>MDM2</i> could be useful for predicting poor treatment outcome in CLL. CLL patients with SNP309 T/G or G/G genotypes had lower overall survival compared with patients harboring the wild-type <i>MDM2</i> gene. The SNPs resulted in higher <i>MDM2</i> expression and subsequently greater deactivation of p53, a tumor suppressor, than was seen in wild-type controls. Next steps include additional validation of the marker in larger patient cohorts and developing agents to target the <i>MDM2</i> -p53 interaction. Ascenta Therapeutics Inc. has three <i>MDM2</i> inhibitors in preclinical testing to treat various types of cancer. Nutlin, a small molecule <i>MDM2</i> antagonist from Roche, is also in preclinical testing to treat cancer.	Not patented; licensing status undisclosed	Gryshchenko, I. <i>et al. J. Clin. Oncol.</i> ; published online May 8, 2008; doi:10.1200/JCO.2007.11.5212 Contact: Inge Tinhofer, Salzburg University Hospital, Salzburg, Austria e-mail: i.tinhofer@salk.at
Glioma	xCT	<i>In vitro</i> and <i>in vivo</i> studies suggest that inhibiting the glutamate transporter xCT could be useful for treating glioma. xCT expression was significantly higher in primary human glioma tumors than it was in healthy human cortical cells ($p < 0.05$). Rats implanted with xCT knockdown gliomas had better survival than those implanted with gliomas expressing xCT. Also, an xCT inhibitor increased the survival of rats with gliomas compared with that of glioma-implanted rats that received vehicle control. In both sets of experiments, knockdown or inhibition of xCT significantly lowered brain edema compared with the effect of control treatment ($p < 0.05$). Next steps include <i>in vivo</i> testing of an xCT-targeted short interfering RNA. (See Swell effect on glioma, page 8.)	Not patented; unlicensed	Savaskan, N. <i>et al. Nat. Med.</i> ; published online May 11, 2008; doi:10.1038/nm1772 Contact: Ilker Eyüpoğlu, University of Erlangen-Nuremberg, Erlangen, Germany e-mail: eyupoglu@gmx.net
Lymphoma	Anaplastic lymphoma kinase (ALK)	Studies in mice suggest that ALK could be a useful antigen for prophylactic or therapeutic vaccines against lymphoma. Vaccination with plasmids encoding portions of ALK protected mice from both local and systemic challenge by ALK ⁺ lymphoma cells, whereas untreated mice developed tumors or systemic disease. The vaccine protected mice for up to seven months by eliciting an ALK-specific interferon- γ response. Combining vaccination with chemotherapy significantly increased survival compared with what was seen using chemotherapy alone ($p < 0.05$). Further studies are necessary to test the vaccination strategy in human cells and then in clinical trials. Novartis AG's ALK inhibitor, NVP-TAE684, is in preclinical testing to treat lymphoma.	U.S. patent application filed for the use of ALK vaccines to cure ALK ⁺ tumors; not yet available for licensing	Chiarle, R. <i>et al. Nat. Med.</i> ; published online May 11, 2008; doi:10.1038/nm1769 Contact: Giorgio Inghirami, University of Torino, Torino, Italy e-mail: giorgio.inghirami@unito.it Contact: Roberto Chiarle, same affiliation as above e-mail: roberto.chiarle@unito.it
Papillary thyroid carcinoma (PTC)	Pre-micro RNA-146a	An <i>in vitro</i> and genotyping study suggests that a SNP in pre-miRNA-146a could be useful for diagnosing PTC. A genotype study of 608 PTC patients revealed that heterozygous expression of the SNP conferred susceptibility to PTC ($p = 7 \times 10^{-6}$), whereas homozygous expression conferred protection from PTC (C/C genotype: $p = 3 \times 10^{-3}$; G/G genotype: $p = 6 \times 10^{-4}$). Next steps include studying a larger number of patients to confirm that PTC predisposition is associated with heterozygosity.	Patent and licensing status undisclosed	Jazdzewski, K. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online May 12, 2008; doi:10.1073/pnas.0802682105 Contact: Albert de la Chapelle, Ohio State University, Columbus, Ohio e-mail: albert.delachapelle@osumc.edu

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cardiovascular disease				
Cardiomyopathy	Cyclic guanosine monophosphate (cGMP)	<p>A study in mice suggests that enhancing cGMP signaling might treat or protect against forms of cardiomyopathy associated with dystrophin (<i>mdx</i>) deficiency. In <i>mdx</i> mouse models of Duchenne muscular dystrophy (DMD), cardiac-specific overexpression of guanylyl cyclase, which catalyzes the production of cGMP, improved cardiac contractility and workload compared with that in hearts from <i>mdx</i> models not overexpressing the enzyme. In six-week-old <i>mdx</i> mice, the phosphodiesterase-5 (PDE-5) inhibitor sildenafil, which prevents cGMP breakdown, improved contractility, energy metabolism and cellular integrity compared with what was seen in untreated controls. Next steps include clinical trials of PDE inhibitors to treat cardiomyopathy.</p> <p>Pfizer Inc. markets Viagra sildenafil, a PDE-5 inhibitor, to treat erectile dysfunction (ED) and pulmonary arterial hypertension (PAH).</p> <p>Other marketed PDE-5 inhibitors include Levitra vardenafil from Bayer AG and Cialis tadalafil from Eli Lilly and Co.</p>	Research not patented; unavailable for licensing	<p>Khairallah, M. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online May 12, 2008; doi:10.1073/pnas.0710595105</p> <p>Contact: Christine Des Rosiers, University of Montreal, Montreal, Quebec, Canada e-mail: christine.des.rosiers@umontreal.ca</p>
Thrombosis; stroke	Activated factor XII (FXIIa)	<p>An SAR study identified 3-carboxamide-coumarins as FXIIa inhibitors that could be useful for treating or preventing thrombosis. FXIIa plays a role in the breakdown of fibrin clots during coagulation. The compounds generally had greater selectivity for but decreased potency against FXIIa compared with the peptidic FXIIa inhibitor Pro-Phe-Arg chloromethyl ketone (PCK). However, PCK is not suitable for oral clinical use as an anticoagulant agent. Next steps include developing oral 3-carboxamide-coumarin analogs with improved FXIIa inhibition and evaluating these compounds as anticoagulant agents.</p>	Not patented; unlicensed	<p>Robert, S. <i>et al. J. Med. Chem.</i>; published online May 7, 2008; doi:10.1021/jm8002697</p> <p>Contact: Lionel Pochet, University of Namur, Namur, Belgium e-mail: lionel.pochet@fundp.ac.be</p>
Endocrine disease				
Metabolic syndrome; type 2 diabetes; obesity	Growth differentiation factor-3 (GDF-3); activin B; activin receptor-like kinase-7 (ALK-7)	<p>Two studies in mice suggest that targeting the TGF-β superfamily receptor ALK-7 and two of its ligands—activin B and GDF-3—could be used to treat hyperglycemia and obesity in metabolic syndrome and type 2 diabetes. ALK-7 knockout mice fed a high-fat diet had higher insulin levels and less weight gain than wild-type mice fed a high-fat diet. Activin B knockouts had insulin levels similar to those seen in ALK-7 knockouts. In contrast, GDF-3 knockout mice fed a high-fat diet had lower weight gain but normal insulin levels compared with wild-type mice fed a high-fat diet. Next steps include using antibodies or soluble ALK-7 fragments to lower the levels of these proteins in mouse models of diabetes or obesity.</p> <p>Acceleron Pharma Inc. is developing soluble fragments of TGF-β superfamily receptors, including ALK-7, to treat type 2 diabetes and obesity. (See Moving upstream of diabetes, page 6.)</p>	Patented; licensed to Acceleron	<p>Andersson, O. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online May 12, 2008; doi:10.1073/pnas.0800272105</p> <p>Bertolino, P. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online May 12, 2008; doi:10.1073/pnas.0800285105</p> <p>Contact: Carlos Ibañez, Karolinska Institute, Stockholm, Sweden e-mail: carlos.ibanez@ki.se</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Type 1 diabetes	IL-2	<p>Studies in cell culture and mice suggest that IL-2 could be useful for treating or preventing type 1 diabetes. In nonobese diabetic mice, multiple low doses of IL-2 restored the balance of CD4⁺/Foxp3⁺ regulatory T cells (Treg) and CD4⁺ effector T cells (Teff) cells, with a consequent slowing or prevention of type 1 diabetes. However, a high dose of IL-2 led to rapid disease onset and enhanced pathogenic function of Teff cells. Next steps include determining the cellular basis both of IL-2 islet deficiency and for toxicity associated with high doses of IL-2 therapy, and developing adjuvant therapy to minimize IL-2 toxicity.</p> <p>Novartis AG markets Proleukin IL-2 to treat metastatic melanoma.</p> <p>At least three other companies have IL-2 therapeutics in development to treat multiple cancers.</p>	Not patented; unlicensed	<p>Tang, Q. <i>et al. Immunity</i>; published online May 8, 2008; doi:10.1016/j.immuni.2008.03.016</p> <p>Contact: Jeffrey Bluestone, University of California, San Francisco, Calif. e-mail: jbluest@diabetes.ucsf.edu</p>
Musculoskeletal disease				
Osteoporosis	Hedgehog (Hh); Patched1 (Ptch1); Gli-Kruppel family member 3 (Gli3); Gli2; repressor form of Gli3 (Gli3rep)	<p>A mouse study suggests that modulating Hh-Ptch1 signaling may be useful for treating osteoporosis. In Ptch1^{-/-} mice, bone mass, bone density and the rate of osteogenesis were significantly higher than what was seen in wild-type mice ($p < 0.05$). Cyclopamine, a steroidal alkaloid that blocks Hh signaling, significantly lowered bone mass and density in wild-type adult mice compared with tomatodine, a steroidal alkaloid that does not block Hh signaling ($p < 0.05$). The study suggests the effects were due to increased Hh signaling and repressed Gli3rep synthesis. Next steps include analyzing Hh-Ptch1 signaling and osteoblast differentiation through Gli2 and identifying Hh agonists that can be delivered specifically to bone tissue.</p>	Patent and licensing status undisclosed	<p>Ohba, S. <i>et al. Dev. Cell</i>; published online May 12, 2008; doi:10.1016/j.devcel.2008.03.007</p> <p>Contact: Ung-il Chung, The University of Tokyo, Tokyo, Japan e-mail: uichung-tky@umin.ac.jp</p> <p>Contact: Shinsuke Ohba, same affiliation as above e-mail: shin-o@umin.ac.jp</p>
Neurology				
Alzheimer's disease (AD)	β -amyloid (A β)	<p>A vaccine expressing a fusion protein consisting of three copies of the β-amyloid (Aβ) epitope, a nonself T helper cell epitope and macrophage-derived chemokine (MDC) as an anti-inflammatory adjuvant could be useful for preventing or treating AD. In a mouse model of AD, vaccination induced a Th2-biased humoral immune response, produced high concentrations of anti-Aβ antibodies and inhibited Aβ accumulation in the brain compared with what was seen in mice immunized with a vaccine expressing a fusion protein consisting of MDC and an irrelevant control antigen. Immunized mice had better spatial memory than control mice. Next steps include safety and immunology studies in larger animals and animal experiments with electroporation device vaccine delivery.</p> <p>At least four companies are developing vaccines that are in preclinical and clinical development to treat AD.</p>	Use of foreign T cell epitope to drive immune response against Ab is patented by Pharmexa A/S; licensed to H. Lundbeck A/S	<p>Movsesyan, N. <i>et al. PLoS ONE</i>; published online on May 7, 2008; doi:10.1371/journal.pone.0002124</p> <p>Contact: Michael G. Agadjanyan, Institute for Molecular Medicine, Huntington Beach, Calif. e-mail: magadjanyan@immed.org</p>
Depression	Adrenergic receptor $\alpha 2$ (ADR $\alpha 2$)	<p>Studies <i>in vitro</i> and in rats suggest that (bis)guanidine and (bis)2-aminoimidazole derivatives could help treat depression. In human prefrontal cortex samples and in rats, two of the derivatives antagonized ADR$\alpha 2$. Enhanced ADR$\alpha 2$ activity has been implicated in depression. Further studies are necessary to determine the toxicity and efficacy of the compounds in animal models.</p> <p>No fewer than seven companies have ADR$\alpha 2$ antagonists in clinical development to treat neurological conditions.</p>	Preliminary patent application filed in Ireland for the antagonists; not yet available for licensing	<p>Rodriguez, F. <i>et al. J. Med. Chem.</i>; published online May 7, 2008; doi:10.1021/jm800026x</p> <p>Contact: Isabel Rozas, University of Dublin, Dublin, Ireland e-mail: rozasi@tcd.ie</p>

This week in techniques

THE DISTILLERY brings you this week's most essential scientific findings in techniques, distilled by *SciBX* editors from a weekly review of more than 400 papers in 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
Computational model for predicting drug toxicity	A predictive model for identifying compounds with off-target activity against the membrane transporter protein multidrug resistance-associated protein 2 (MRP2) could help exclude potentially toxic drug candidates from the early stages of drug discovery. Inhibition of MRP2 can disrupt hepatic lipid homeostasis and cause liver toxicity. The computational model discriminated inhibitors from noninhibitors based solely on molecular structure, with predictive powers of 86% and 72% for two different sets of compounds. MRP2 inhibitors were generally larger and more hydrophobic, and they had higher aromaticity and better hydrogen binding capacity than noninhibitors. Next steps include comparing modes of MRP2 inhibition with those of other membrane transporters.	Not patented; unlicensed	Pedersen, J.M. <i>et al. J. Med. Chem.</i> ; published online May 6, 2008; doi:10.1021/jm7015683 Contact: Per Artursson, Uppsala University, Uppsala, Sweden e-mail: per.artursson@farmaci.uu.se
Fluorescence microscopy for 3D imaging of cells	Biplane fluorescence photoactivation localization microscopy (BP FPALM) could be useful for detecting subcellular disease-associated processes that were previously detectable only by electron tomography (sectional imaging). The method imaged layers of 40 nm diameter fluorescent beads at a resolution of about 30 nm laterally and 75 nm axially, which is superior to the 100 nm resolution of 3D light microscopy. The BP FPALM technology is compatible with live-cell imaging. Next steps include developing photosensitive imaging markers and probes.	Two patent applications filed for 3D FPALM; available for licensing from The Jackson Laboratory	Juette, M.F. <i>et al. Nat. Methods</i> ; published online May 11, 2008; doi:10.1038/nmeth.1211 Contact: Joerg Bewersdorf, The Jackson Laboratory, Bar Harbor, Maine e-mail: joerg.bewersdorf@jax.org
High throughput protein microarray	<i>In vitro</i> studies suggest that a nucleic acid programmable protein array (NAPPA) could be useful for identifying therapeutic targets in multiple diseases. The method allowed for functional proteins to be synthesized directly from printed complementary DNAs, resulting in high-density arrays of hundreds of gene products. Protein displays were reproducible between replicates within an array and between duplicate arrays. Next steps include evaluating the utility of the method for detecting protein-protein interactions, screening enzyme substrates and measuring small molecule binding kinetics.	NAPPA technology has multiple patents; available for licensing through Auguron Biosciences Inc.	Ramachandran, N. <i>et al. Nat. Methods</i> ; published online May 11, 2008; doi:10.1038/nmeth.1210 Contact: Joshua LaBaer, Harvard Medical School, Cambridge, Mass. e-mail: joshua_labaer@hms.harvard.edu
Nanoparticle drug delivery of chemotherapeutics	Studies <i>in vitro</i> and in xenograft mice suggest that nanoparticle-mediated delivery of paclitaxel prodrugs could offer improved activity over commercial formulations of paclitaxel. The prodrugs were formulated in polymer-stabilized nanoparticles, and the rate of prodrug release could be adjusted by varying the hydrophobicity of the prodrug's lipid anchor. Next steps include toxicology studies of the nanoparticle prodrugs. Paclitaxel is a generic chemotherapeutic that is marketed to treat multiple types of cancer. No fewer than 24 companies have paclitaxel-based compounds and delivery devices in development stages ranging from preclinical to marketed.	Patent applications filed for the nanoparticle technology; available for licensing	Ansell, S. <i>et al. J. Med. Chem.</i> ; published online May 9, 2008; doi:10.1021/jm800002y Contact: Steven M. Ansell, Celator Pharmaceuticals Corporation, Vancouver, British Columbia, Canada e-mail: sansell@celatorpharma.com
Self-assembled artificial viruses for drug delivery	An artificial filamentous viral assembly could offer improved bioavailability compared with that of conventional carriers for delivery of gene therapy or encapsulated molecules. As proof of concept, the viral assembly transfected HeLa cells with GFP short interfering RNA with efficiency comparable to that of Lipofectamine 2000 (LF2000), an RNA transfection reagent marketed by Invitrogen Corp. The viral assembly also delivered molecules of the dye Nile red to both the cytoplasm and nucleus of the HeLa cells. Next steps include refining the technique to incorporate additional functional properties of natural virions into the artificial ones, such as endosome escape, efficient cell binding, nucleus localization and tissue targeting.	Not patented; licensing status not applicable	Lim, Y.-B. <i>et al. Angew. Chem. Int. Ed.</i> ; published online May 7, 2008; doi:10.1002/anie.200800266 Contact: Myongsoo Lee, Yonsei University, Seoul, Korea e-mail: mslee@yonsei.ac.kr

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