

THIS WEEK**ANALYSIS****COVER STORY****1 Spine fix for ALS**

Q Therapeutics is aiming to begin human trials for targeted transplantation of astrocyte precursor cells to slow the loss of respiratory function in ALS, building on animal proof of concept published by a team of collaborators from Johns Hopkins and Invitrogen Corp.

TARGETS & MECHANISMS**4 A tolerant alternative to immunosuppression**

Two teams of researchers have shown that short-term α_1 -antitrypsin monotherapy can lead to long-term survival of islet β -cells following transplantation—without the need for immunosuppressants. Both groups are already planning human trials of AAT in type 1 diabetes.

6 The long and short of fat

U.S. researchers have shown how modulating two free fatty acid receptors may influence fat absorption. With patent protection for Roche's Xenical orlistat set to expire in 2009, companies looking for new ways to block fat absorption without some of the drug's side effects are paying attention.

8 Gassing hypertension

U.S. and Canadian researchers have shown that endogenous hydrogen sulfide gas can regulate blood pressure. Although the findings reveal potential new intervention points in hypertension, how to apply them therapeutically while balancing efficacy and toxicity remains an open question.

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By Michael J. Haas, Senior Writer

Researchers have shown in *Nature Neuroscience* that targeted transplantation of astrocyte precursor cells may extend the lifespan of patients with amyotrophic lateral sclerosis by slowing the loss of respiratory motor neuron function.¹ **Q Therapeutics Inc.** and some of the researchers are collaborating to develop the findings for ALS and other indications involving spinal cord degeneration.

ALS causes loss of sensation, loss of motor control and paralysis. Patients usually die three to five years after diagnosis, when degeneration of motor neurons leads to respiratory failure. Rilutek riluzole, a glutamate antagonist from **sanofi-aventis Group**, is the only approved ALS drug.

Studies over the last seven years have suggested that diseased non-neuronal cells such as astrocytes contribute to neuronal dysfunction and death in both forms of the disease. Familial ALS occurs in about 20% of patients, and about one-fourth of such cases are linked to mutations in the gene coding for superoxide dismutase 1 (SOD1). Nonfamilial, sporadic ALS is the most common form.

In February, researchers at the **University of California, San Diego** reported that loss of motor neuron function was slower in mice whose astrocytes did not express mutant SOD1 than it was in control mice expressing mutant SOD1, confirming that astrocytes do play a role in the progression of ALS.²

The problem, however, is that ALS affects the entire spinal column, and as a result cell-based therapies have been deemed unfeasible, according to Jeffrey Rothstein, coauthor of the *Nature Neuroscience* paper. Rothstein is a professor of neurology and neuroscience and co-director of the Brain Science Institute at **Johns Hopkins University**.

"Instead of trying to treat the entire spinal column, we targeted the part of the spinal column that involves the respiratory motor neurons to address the main cause of mortality in ALS," he told *SciBX*.

The group was led by Nicholas Maragakis, associate professor of neurology at Johns Hopkins, and also included Mahendra Rao, who is cofounder and chief scientific consultant at Q Therapeutics and VP of stem cell research at **Invitrogen Corp.** In 1998, Rao and other researchers at the **University of Utah School of Medicine** developed the rodent astrocyte precursor cells.³

For their study, the Maragakis team used rodent cells called glial-restricted precursor cells, which can differentiate into only two types of cells: astrocytes, which support the functions of nearby neurons, or oligodendrocytes, which regulate neuron myelination.

The team from Johns Hopkins and Invitrogen reported that astrocyte precursor cells injected at multiple sites on the spines of rats with ALS

A tolerant alternative to immunosuppression

By Kai-Jye Lou, Staff Writer

Two research teams have shown that temporary monotherapy with human α_1 -antitrypsin can lead to long-term protection against inflammation-mediated and T cell-mediated destruction of islet β -cells in mice. The results, which were achieved without the use of immunosuppressants, could bolster the prospects for islet transplantation in patients with type 1 diabetes by overcoming two of the main drawbacks associated with such therapy: immunosuppressant use and eventual loss of graft function.

Both groups are already planning clinical trials to evaluate AAT in type 1 diabetics.

Human α_1 -antitrypsin (SERPINA1; AAT) is an anti-inflammatory serine protease inhibitor that is marketed as an enzyme replacement therapy for patients with a defective version of the protein (AAT deficiency). The FDA has approved three AAT products: Aralast from **Baxter International Inc.**, Prolastin from **Talecris Biotherapeutics Inc.** and Zemaira from **sanofi-aventis Group**.

In a paper published in the *Proceedings of the National Academy of Sciences*, researchers at the **University of Colorado Health Sciences Center** and **Ben-Gurion University of the Negev** showed that AAT monotherapy resulted in dose-dependent increases in acceptance rates of allogeneic islet β -cell grafts and normal glucose levels in diabetic mice.¹ The grafts persisted for the duration of the 12-week experiment, whereas all grafts in albumin-treated controls were rejected within 12 days.¹

The research group was led by Charles Dinarello, a professor of medicine at the University of Colorado.

The accepted grafts remained functional and maintained normal glucose levels following cessation of AAT, whereas removal of the grafts led to hyperglycemia. Importantly, mice receiving a second islet graft from the same source, following removal of the initial graft, did not require additional doses of AAT for immune tolerance. In contrast, mice receiving islet β -cells from a different source quickly rejected the second graft—a result that suggests the induced immune tolerance of grafted cells is specific to the original tissue source.

Separately, researchers at **Harvard Medical School** published in *PNAS* that AAT monotherapy halted the autoimmune reactions that lead to the destruction of native islet cells. The treatment also partially restored native islet β -cell mass and normal glucose levels in 14 of 16 nonobese diabetic (NOD) mice, a significant improvement compared with what was seen in the 150 untreated controls, which all remained hyperglycemic ($p < 0.0001$).²

The research group was led by Terry Strom, scientific director of the Transplant Institute at **Beth Israel Deaconess Medical Center** and a professor of medicine at Harvard Medical School.

“Clearly AAT not only alters autoimmunity but also transplant immunity.”

—Terry Strom,
Beth Israel Deaconess
Medical Center

Similar to Dinarello's group, Strom's team showed that diabetic mice receiving AAT and islet β -cells from a syngeneic source accepted the graft and had normal glucose levels. Grafts in untreated controls were destroyed.

Both *PNAS* papers showed that AAT does not directly affect T cells. Instead, AAT shifts the body's cytokine balance from proinflammatory to anti-inflammatory. The anti-inflammatory environment prevents the maturation of dendritic cells (DCs) and encourages the conversion of naïve T cells into protective T regulatory cells that prevent effector T cells from attacking the islet β -cells.

Lowering the number of mature DCs also downregulates the conversion of naïve T cells into effector T cells (see **Figure 1, “Protecting islet cell grafts using AAT”**).

“The most important finding is that we do not see dendritic cells mature—they process foreign antigens from the allograft but present them to naïve T cells in an immature state, thus inducing antigen-specific tolerance,” Dinarello told *SciBX*.

Strom noted that his team became interested in this area after Dinarello shared some initial observations; he thinks the results reported in *PNAS* by Dinarello's group complement the work from his own group. “Clearly AAT not only alters autoimmunity but also transplant immunity. The underlying mechanism in both cases appears to involve a beneficial modification of the inflammatory milieu in which autoimmunity or transplant immunity is spawned,” Strom told *SciBX*.

Away with immunosuppression

Strom and Dinarello also noted that the results reported in the *PNAS* articles were achieved without the use of potentially toxic immunosuppressant drugs. Normally, patients receiving transplanted islet cells would need to take an immunosuppressant cocktail for the life of the graft.

NIH's National Institute of Diabetes and Digestive and Kidney Diseases warns that the use of immunosuppressive drugs in patients receiving pancreatic islet transplantation is known to increase both susceptibility to infections and cancer risk.

Moreover, a paper in *Diabetes* by Canadian researchers showed that only about 10% of islet cell transplant recipients using the standard immunosuppressant drug cocktail remained free of the need for insulin injections at a five-year follow-up.³

Thus, said Robert Elliot, medical director and cofounder of **Living Cell Technologies Ltd.**, “immunosuppression has yielded few results” from the attempts to treat type 1 diabetes “along the immune track.”

Living Cell's DiabeCell encapsulated porcine pancreatic islet cells are in Phase I/II testing. The capsules are intended to ensure the cells are not recognized as foreign by the recipient's immune system, thus circumventing the need for immunosuppressants.

“All drugs currently approved to prevent transplant rejection are immunosuppressive, but the real problem is that these drugs are also toxic,” said Dinarello. “AAT does not suppress the immune system, so you don't have the standard problems associated with immunosuppressive drugs.”

Eli Lewis, a lead author on the Dinarello paper and director of the

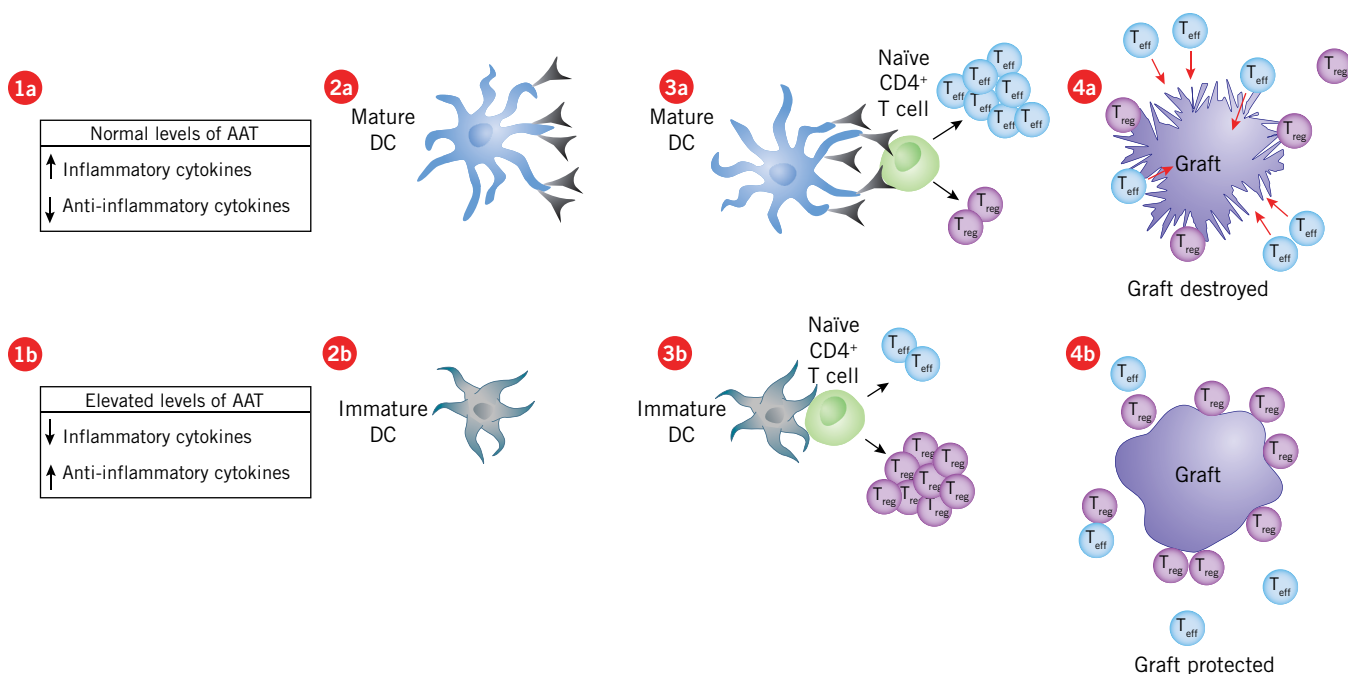


Figure 1. Protecting islet cell grafts using AAT. Human α_1 -antitrypsin (SERPINA1; AAT), which does not directly act on T cells, has anti-inflammatory properties that are believed to promote host immune tolerance toward islet β -cell grafts by expanding the population of T regulatory (T_{reg}) cells and decreasing the number of effector T (T_{eff}) cells.

Two papers in *PNAS*^{1,2} now show that treatment with AAT can shift the cytokine environment from proinflammatory [1a] to anti-inflammatory [1b].

Immature dendritic cells (DCs) collect antigens from the islet β -cells. In a proinflammatory environment, DCs will mature and express an array of surface molecules that encourage the conversion of naïve T cells into T_{eff} cells [2a]. When inflammation is blunted by the addition of AAT, the DCs remain in an immature state and fail to express the surface molecules needed for T_{eff} cell conversion [2b].

The antigen-bearing DCs engage naïve T cells and imprint them to recognize the foreign antigen. When engaged with a mature DC, a naïve T cell is encouraged to differentiate into a T_{eff} cell [3a]. When engaged with an immature DC, the naïve T cell will tend to differentiate into a T_{reg} cell [3b].

Without a sufficient population of antigen-specific T_{reg} cells, the T_{eff} cells invade and kill the islet β -cells [4a]. However, when sufficient T_{reg} cells physically reside in the vicinity of the islet β -cells, they disarm the incoming T_{eff} cells [4b]. In addition, subsequent islet β -cell grafts from the same donor may be accepted without the need for repeated treatment, as demonstrated in the two *PNAS* papers.

Clinical Islet Lab at Ben-Gurion University of the Negev, noted that “it is quite unique that a genuinely anti-inflammatory molecule was able to re-edit the immune system to achieve antigen-specific tolerance mediated by T regulatory cells.” Immunosuppressive therapy, he said, only delays the immune system from recognizing the foreign tissue.

“The most surprising aspect is that the AAT can redirect, through changes in inflammation, the activity of tissue-destructive T cells toward a tissue-protective mode,” said Strom.

Easy dose it

Even if AAT can take immunosuppressants out of the picture, a question going forward is whether—and at what dose—AAT will be able to improve on the success rates for islet cell transplant therapies.

“The five-year islet graft survival rates have been reported as being very low” in humans, noted Lewis. “We are hoping that the behavior

of the mouse immune system, especially chosen in our study to be that of healthy wild-type strains, reflects that of humans. We believe only a clinical trial can provide the answer, a particularly feasible option considering the safety record of AAT.”

He added: “We chose AAT for a reason. Aside from being such a powerful anti-inflammatory agent, it has been used in patients for over two decades as enzyme replacement therapy. Studies for up to 13 years clearly show that these patients exhibit no compromise in their inflammatory responses.”

In the diabetes setting, Lewis said, a few weeks of AAT treatment “appears to be sufficient for graft acceptance and induction of immune tolerance.”

Nevertheless, Ingrid Stuijver, senior director of research at **MicroIslet Inc.**, cautioned against directly translating results from murine models to humans.

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The long and short of fat

By Lev Osherovich, Senior Writer

With patent protection for Roche's Xenical orlistat set to expire in 2009, the obesity drug's \$531 million in 2007 sales will soon be up for grabs. Now, two mouse studies make a case for blocking receptors for free fatty acids—short chain in one case and long chain in the other—that are downstream of Xenical's target. Although some drug developers already have explored brain- and pancreas-specific homologs of one of these receptors to treat metabolic diseases, hitting the intestinal version could bypass some of Xenical's side effects while still blocking fat absorption.

Xenical works by blocking pancreatic lipase, an enzyme secreted into the intestine that liberates dietary fatty acids from a triglyceride precursor form, causing triglycerides to pass through the digestive tract.

The new studies show that fatty acids can also engage surface receptors that influence fatty acid transport, metabolism and appetite.

One study focused on modulating free fatty acid receptor 3 (FFAR3; GPR41), a receptor for short-chain fatty acids like acetate and propionate that are produced by intestinal microbes.¹ The second study described how a receptor called CD36 molecule (thrombospondin receptor)

(SCARB3; FAT; CD36) senses oleate, a long-chain fatty acid, thus stimulating a pathway leading to satiety.²

Short and sweet

The GPR41 study was published in *Proceedings of the National Academy of Sciences* by a team led by Masashi Yanagisawa, professor of molecular genetics at the **University of Texas Southwestern Medical Center**, and Jeffrey Gordon, director of the Center for Genome Sciences at the **Washington University School of Medicine**.

The work builds on earlier efforts to characterize the location and function of GPR41 and its close relatives, GPR40 and GPR43. Because the ligands for this receptor family were recognized as dietary fatty acids, the proteins became interesting obesity targets for at least two companies.

Modulating GPR40 and GPR43 activity already has been attempted by **GlaxoSmithKline plc** and **Amgen Inc.**, according to published studies.^{3,4} A GSK spokesperson told *SciBX* the company's project in this area is no longer active for undisclosed reasons. Amgen's GPR43 program also is no longer active, according to a company spokesperson.

But whereas GPR40 and GPR43 are found in multiple tissues, including the brain and pancreas, Yanagisawa and Gordon's team discovered that GPR41 was expressed primarily in the enteroendocrine cells of the intestinal ileum and colon.

Thus, as an intestinal surface protein, GPR41 may in fact be more druggable than its counterparts in the brain and pancreas.

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"Studies in several different animal models such as the diabetes-prone BB [BioBreeding] rat or nonhuman primates might be enlightening," she told *SciBX*. "We would like to see the preclinical work include a larger subset of animals to assure reproducibility."

The company is planning its IND submission to enter the clinic with its MicroIslet-P microencapsulated porcine islet cell implant.

Maria Koulmanda, a lead author on the Harvard *PNAS* paper, also noted AAT potentially can induce T cell tolerance toward harmful pathogens. Given the potential for pathogen tolerance, she said the clinical trial protocol involving AAT therapy would specifically exclude patients with active infections.

Koulmanda, is associate professor of surgery at Harvard and director of nonhuman primate research at Beth Israel Deaconess Medical Center.

AAT designs

Dinarello said his group is planning a clinical trial to evaluate AAT in patients with type 1 diabetes undergoing islet cell transplantation. "The real question is how long you have to give AAT," Dinarello told *SciBX*.

Similarly, Strom's group is working on clinical trial designs for AAT-based therapies to treat new onset type 1 diabetes.

The University of Colorado has a patent covering inhibitors of serine protease activity and their use in treatment of graft rejection and promotion of graft survival. The work is available for licensing from the University of Colorado Office of Technology Transfer.

Strom said a patent application has been filed covering the use of

AAT in type 1 diabetes and autoimmune diseases and is available for licensing from the Technology Ventures Office at Harvard.

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Published online Nov. 6, 2008

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

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

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Autoimmune disease				
Islet cell transplant; type 1 diabetes	Serine protease	<p>A study in mice suggests that α_1-antitrypsin (AAT) may be useful for inducing immune tolerance to transplanted islet cells. In diabetic mice receiving allogeneic islet cell grafts, AAT increased graft survival, whereas all grafts in albumin-treated controls were rejected. In mice that received a second same-strain islet graft following the removal of the initial one, immune tolerance was maintained without AAT. Next steps include clinical trials of AAT to prevent transplant rejection.</p> <p>Baxter International Inc. markets Aralast hAAT to treat congenital AAT deficiency in patients with clinically evident emphysema. Otelixizumab, an mAb that binds to CD3 from Tolerx Inc., BTG plc and GlaxoSmithKline plc, is in Phase III testing to treat type 1 diabetes. DiaPep277, an immunomodulator peptide analog of a heat shock protein 60 epitope from Clal Biotechnology Industries Ltd. and Teva Pharmaceutical Industries Ltd., is in Phase III testing for the same indication.</p> <p>At least six additional companies have immune modulators or cell therapy products in Phase II or earlier to treat type 1 diabetes (<i>see A tolerant alternative to immunosuppression, page 4</i>).</p> <p>SciBX 1(40); doi:10.1038/scibx.2008.966 Published online Nov. 6, 2008</p>	Patent owned covering inhibitors of serine protease activity and their use in treatment of graft rejection and promotion of graft survival; available for licensing from the University of Colorado Office of Technology Transfer	<p>Lewis, E.C. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Oct. 13, 2008; doi:10.1073/pnas.0807627105</p> <p>Contact: Charles A. Dinarello University of Colorado Health Sciences Center Denver, Colo. e-mail: cdinarello@mac.com</p>
Cancer				
Cancer	BCL2-like 1 (BCL-X _L ; BCL2 _{L1})	<p>SAR studies identified analogs of chelerythrine and sanguinarine that inhibit BCL-X_L and could be optimized to treat cancer. A fluorescence polarization assay identified three newly synthesized analogs of chelerythrine and five analogs of sanguinarine with more potent BCL-X_L binding and inhibitory activity than their respective parent compounds. The most potent analogs bound to different sites on the apoptosis-promoting protein, with the sanguinarine compounds binding to the BCL2 homology domain 1 and the chelerythrine compounds binding to the BCL2 homology domain groove. Additional studies are necessary to further enhance the potency and solubility of the compounds.</p> <p>SciBX 1(40); doi:10.1038/scibx.2008.967 Published online Nov. 6, 2008</p>	Compounds unpatented; unavailable for licensing	<p>Bernardo, P. <i>et al. J. Med. Chem.</i>; published online Oct. 17, 2008; doi:10.1021/jm8005433</p> <p>Contact: Christina L.L. Chai, Agency for Science Technology and Research (A*STAR), Jurong Island, Singapore e-mail: christina_chai@ices.a-star.edu.sg</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Type 1 diabetes	Serine protease	<p>A study in mice suggests that α_1-antitrypsin (AAT) may be useful for treating type 1 diabetes. In 14 of 16 nonobese diabetic (NOD) mice, AAT helped maintain euglycemia, whereas 150 untreated controls remained hyperglycemic ($p < 0.0001$). In NOD mice receiving syngeneic islet grafts, AAT induced immune tolerance of the graft and euglycemia, whereas grafts in untreated controls were destroyed. Next steps include clinical trials of AAT to treat type 1 diabetes and testing AAT with immunosuppressants in organ transplantation procedures.</p> <p>Baxter International Inc. markets Aralast hAAT to treat congenital AAT deficiency in patients with clinically evident emphysema. Otelixizumab, an mAb that binds to CD3 from Tolerx Inc., BTG plc and GlaxoSmithKline plc, is in Phase III testing to treat type 1 diabetes. DiaPep277, an immunomodulator peptide analog of a heat shock protein 60 epitope from Clal Biotechnology Industries Ltd. and Teva Pharmaceutical Industries Ltd., is in Phase III testing for the same indication.</p> <p>At least six additional companies have immune modulators or cell therapy products in Phase II or earlier to treat type 1 diabetes (<i>see A tolerant alternative to immunosuppression, page 4</i>).</p> <p>SciBX 1(40); doi:10.1038/scibx.2008.974 Published online Nov. 6, 2008</p>	<p>Patent application filed for use of human AAT in type 1 diabetes and autoimmune diseases; available for licensing from Harvard University Technology Ventures Office</p> <p>Contact: Mark Chalek, Beth Israel Deaconess Medical Center, Boston, Mass. e-mail: mchalek@bidmc.harvard.edu</p>	<p>Koulmanda, M. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Oct. 13, 2008; doi:10.1073/pnas.0808031105</p> <p>Contact: Terry B. Strom, Beth Israel Deaconess Medical Center, Boston, Mass. e-mail: tstrom@bidmc.harvard.edu</p>
Inflammation				
Inflammatory disease	Complement component 3 (C3); complement component 5 (C5); IL-8 (CXCL8)	<p>Studies in cell culture suggest that the Staphylococcal superantigen-like 5 (SSL5) protein may be useful in treating disorders characterized by excessive recruitment of leukocytes, such as inflammatory diseases. In cultured neutrophils, SSL5 prevented leukocyte activation induced by C3, C5 and CXCL8 in a dose-dependent manner. In cellular assays, SSL5-treated cells did not show signs of toxicity. Next steps include identifying a nonimmunogenic SSL5 analog for use in humans.</p> <p>SciBX 1(40); doi:10.1038/scibx.2008.975 Published online Nov. 6, 2008</p>	<p>Patent pending for use in inflammatory diseases mediated by neutrophil influx such as reperfusion injury; available for licensing</p>	<p>Bestebroer, J. <i>et al. Blood</i>; published online Oct. 21, 2008; doi:10.1182/blood-2008-04-153882</p> <p>Contact: Jovanka Bestebroer, University Medical Center Utrecht, Utrecht, the Netherlands e-mail: j.bestebroer@umcutrecht.nl</p>