

THIS WEEK

ANALYSIS

COVER STORY

1 Metastasis, interrupted

Two research teams have identified two molecules that could play key roles in metastasis—CD151 as a potential target to prevent metastatic tumors and SATB1 as a potential target to prevent or treat metastasis. Their differences illustrate that efforts to prevent metastatic processes would be difficult to demonstrate in the clinic. Meanwhile, the druggability of each target has yet to be established. For CD151, the issue is that hitting the target may be too late in the metastatic process. SATB1, on the other hand, is found only in the nucleus and thus could have both accessibility and side effect issues.

TARGETS & MECHANISMS

5 Staph: the NOse have it

A study in *Science* solves the mystery of why *S. aureus* thrives in the nose, which is full of immune cells and antimicrobial molecules that kill most other bacteria. But inhibiting the bacterial target could have unknown knock-on effects in humans.

6 Putting cancer to REST

Two *Nature* papers lay out the machinery responsible for regulating REST, a zinc-finger DNA-binding protein that can either induce or ward off tumors. One key target could be an E3 ubiquitin ligase that mediates the degradation of REST in a variety of cell types. However, blocking the protein-protein interaction is a challenging task for small molecules.

THE DISTILLERY

9 This week in therapeutics

Treating Alzheimer's disease with cholinesterase inhibitors; controlling sepsis with CD44 inhibitors; using IL-15 as an adjuvant; reducing obesity via cannabinoid receptor antagonists; diagnosing leukemia with microRNAs; treating cancer with CD32B; and more...

13 This week in techniques

In vivo delivery of microRNA antagonists; *in silico* identification of efficacious drug combinations; virtual screening of GPCR antagonists; and more...

INDEXES

13 Company and institution index

14 Target and compound index

Metastasis, interrupted

By Michael J. Haas, Senior Writer

Two studies have clarified the roles of two different proteins in metastasis, suggesting potential new therapeutic strategies to treat cancer. One study proposes agonizing the membrane protein CD151 to prevent cancer cells from mobilizing at primary tumors;¹ the other suggests turning off special AT-rich sequence binding protein 1 (SATB1), a regulatory master switch that promotes cancer cell invasiveness and metastasis.²

Company researchers contacted by SciBX agreed that both papers uncovered important molecular mechanisms that drive metastasis, but they were less certain that CD151 and SATB1 are druggable targets. Druggability concerns aside, the companies suggested it might be easier to demonstrate outcomes in the clinic by targeting SATB1 rather than CD151.

Not letting go

In their paper in *Cancer Cell*, researchers at **The Scripps Research Institute** and **The Italian Foundation for Cancer Research Institute for Molecular Oncology** report that agonizing the membrane protein CD151 promotes tumor cell immobility at the primary tumor site and prevents the cells from migrating.¹ The team was led by James Quigley, a professor of cell biology at Scripps.

This work built on previous *in vitro* studies conducted at the **State University of New York at Stony Brook** by Quigley and colleagues, showing that tetraspanin CD151 plays a role in tumor migration and metastasis.³

Tetraspanins are membrane proteins thought to anchor other proteins to the cell membrane. CD151, also known as tetraspanin 24, is a cell-surface signal transducer involved in cell development, activation, growth and motility. It complexes with integrins and other tetraspanins and is believed to regulate the functions of these proteins. CD151 is over-expressed in many cancers.

In the current paper, chick embryos and mice with fluorophore-labeled human tumors were injected with 1A5, an anti-CD151 mAb previously developed by Quigley at SUNY Stony Brook.⁴

Tumor cells were tracked with **Innovascreen Inc.**'s intravital imaging technology—a general term for methods that image live tissue, according to the company's CEO, John Lewis. He said Innovascreen has improved intravital technology by developing the chick embryo model—which extends the real-time imaging timeframe from hours to days—and by incorporating new fluorescence techniques and instrumentation to increase resolution and overall utility.



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Quigley's team found that 1A5 prevented tumor cells from detaching and migrating into the bloodstream—a process called intravasation. 1A5 did not prevent tumor cells from leaving the bloodstream and entering new tissues (extravasation), but it did prevent them from migrating through those tissues afterwards.

The mAb had no effect on the motility of CD151-deficient tumor cells. Nor did the antibody have an effect on tumor growth and proliferation, although it prevented primary tumor cells from invading the surrounding tissue.

The findings indicate that 1A5 stimulates the natural adhesion-promoting function of CD151, thereby inhibiting tumor cell motility and blocking the first step of metastasis.

Andries Zijlstra, spokesperson for the team, said the natural ligand of CD151 and the exact mechanism by which CD151 promotes adhesion are unknown. What is known is that CD151 interacts with integrin $\alpha_3\beta_1$ (VLA-3) and integrin $\alpha_6\beta_1$. Thus, a possible explanation is that CD151 regulates adhesion and migration by direct control of integrin function.

Formerly at Scripps, Zijlstra is now an assistant professor of pathology and cancer biology at **Vanderbilt University**.

The question is whether the CD151 findings can be translated into antimetastatic therapies, given the problems with designing clinical trials that show prevention of metastasis.

"It has long been hypothesized in the metastasis community that there should be some way to prevent tumor cells from leaving the primary tumor," said David Young, president and CEO of **Arius Research Inc.** "So it's interesting to find a molecule that regulates this process. But the relevance to drug development is quite challenging" because of the difficulties of testing whether a compound prevents metastasis.

"You are trying to prove a negative—that metastasis hasn't occurred," he said. "The population size and long follow-up time required to study an antimetastatic drug compound can be problematic."

Arius has two compounds in preclinical development: AR001, a mAb targeting CD44 on cancer stem cells, and AR002, a mAb targeting the signal transducer tumor-associated calcium signal transducer 2 (TACSTD2; TROP2). The company plans to start Phase I trials of both compounds to treat solid tumors in late 2008.

Another issue, said Young, is the lack of data on the mechanism by which CD151 promotes adhesion. "It might not be the culprit molecule. It might interact with something else that is the dominant player. CD151 might be the organizer factor, not the primary effector" of the adhesion process, he said.

"Also, they haven't shown the other half of it—increased survival in connection with decreased metastasis in the animals," Young said. Such a correlation could provide a measurable endpoint for clinical development and possibly show whether CD151 is indeed the governing molecule in the process, he said.

Andrew Mazar, CSO and SVP of R&D at **Attenuon LLC**, thinks anti-CD151 therapies may be too little, too late. "The problem here is that the research team is looking at a process that leads to intravasation," he said. "But by the time a primary tumor is macroscopically detectable, it may well already have intravasated and metastasized."

Attenuon's ATN-161 is a five-residue peptide derived from fibronectin that targets integrin $\alpha_3\beta_1$ and integrin $\alpha_v\beta_3$. In mid-2007 the company halted a Phase II trial of the compound in intracranial malignant glioma because of the inconvenience of the i.v. formulation. Attenuon is

reformulating the compound for subcutaneous injection and expects to re-enter the clinic within 18 months.

Tim Hoey, VP of cancer biology at **OncoMed Pharmaceuticals Inc.**, thinks the findings would be difficult to develop clinically because targeting CD151 did not affect growth of the primary tumor. “If the antimetastatic agent didn’t affect the primary tumor, it would be harder to follow its effects. You would have to do a trial around long-term survival or absence of metastasis—endpoints that take years to play out. That’s quite a gamble of time and expense for proof of concept.”

OncoMed’s OMP-21M18 mAb against an undisclosed target on cancer stem cells is expected to start a Phase I trial in solid tumors in late 2008. The compound is partnered with **GlaxoSmithKline plc**.

“It is usually necessary to show some effect on the tumor,” Young noted. “And an antimetastatic drug is not likely to be chosen as a first-line treatment for early-stage patients—even though they would most likely benefit from it.”

Zijlstra acknowledged it is difficult to predict when metastasis will occur, but disagreed that targeting the CD151-mediated mobilization of tumor cells would have no effect on the primary tumor.

“This mobilization is not only important for metastasis,” he said. “It is also responsible for the local invasion of host tissue that characterizes a malignant tumor.”

Thus, immobilizing tumor cells might prevent metastasis and revert the malignant phenotype of a primary tumor. “This is particularly important for invasive tumors of critical organs such as pancreatic and brain cancers,” which are not metastatic in the classic sense because the tumor cells haven’t migrated to distant sites, said Zijlstra.

Such tumors might benefit from a therapy like the anti-CD151 mAb, he said, because “these invasive structures are difficult, if not impossible, to remove surgically.”

Zijlstra said the research team is investigating potential adhesion and de-adhesion partners for CD151. “We have found about 50 of them and are looking at which ones result in the immobile phenotype” in cancer cells and might provide better targets than CD151, he said.

The research team also is conducting a “study that correlates CD151 and its partners to disease progression and survival,” Zijlstra said.

Metastatic master switch?

Meanwhile a paper in *Nature* by researchers at the University of California’s **Lawrence Berkeley National Laboratory** and **Fox Chase Cancer Center** reports that SATB1 is expressed in breast cancer cells and deregulates the expression of many proteins, including cell adhesion molecules that enable tumor cell migration.² The research team, led by Terumi Kohwi-Shigematsu, senior scientist at Lawrence Berkeley, suggests that SATB1 inhibitors could be used to treat or even prevent metastatic breast cancer.

Companies and institutions contacted by *SciBX* said the results on SATB1 may hold greater promise than CD151 for drug development. However, the consensus is that targeting SATB1 directly will still be difficult and could have far-reaching side effects.

SATB1 is a nuclear protein that regulates chromatin—the intertwined DNA and proteins that make up chromosomes. SATB1 forms cage-like

structures that fold and remodel chromatin to coordinate gene expression and regulation. It is also expressed on normal, activated T cells.

Kohwi-Shigematsu and her team examined 24 breast epithelial cell lines and detected SATB1 expression only in cells known to be aggressive and metastatic. The team also detected SATB1 in aggressive types of human primary breast tumors, but not in adjacent, nonmalignant tissue.

“Normal epithelial breast cells do not express SATB1 at all,” she told *SciBX*. “Many cancer cells do not express SATB1 either. SATB1 expression is closely associated with aggressive and metastatic cancer cells.”

The team next showed that short hairpin RNA knockdown of SATB1 reversed the metastatic activity of metastatic breast cancer cells *in vitro* and in mice. They also showed that abnormal expression of SATB1 in mice with ordinarily nonmetastatic tumor cells induced the development of invasive, metastatic tumors.

Thus, the team concluded that expression of SATB1 was key to the metastatic phenotype of tumor cells. “Our results suggest that SATB1 is induced during cancer progression, and once SATB1 is expressed, cells metastasize,” Kohwi-Shigematsu said.

Gene expression profiling of breast tumor cells revealed that SATB1 upregulated or downregulated more than 1,000 genes. The largest proportion corresponded to genes that code for cell adhesion molecules.

Among the upregulated molecules were three that are often seen in invasive breast cancer: OB-cadherin (cadherin-11), VE-cadherin (CD144; cadherin-5) and N-cadherin. Among the downregulated adhesion molecules was the junction protein and tumor suppressor E-cadherin, which helps normal cells stay in place.

Taken together, Kohwi-Shigematsu said the data strongly suggest that breast cancer metastasis results from expression of SATB1, which

in turn alters the expression profile of many genes to promote metastatic activity and tumor growth. Thus, targeting SATB1 in tumor cells could reverse the aggressive phenotypes of metastatic breast cancer, she said.

Arius’s Young was interested in the correlation Kohwi-Shigematsu’s team found between SATB1 and tumor growth and metastasis, but would need to know more about the role SATB1 plays in normal tissues before deciding whether it is a druggable target. The researchers “haven’t done a lot to characterize the expression of SATB1 in normal cells and tissues,” which raises potential toxicity issues when targeting the protein, he said.

Young added that the genetic regulatory functions of SATB1 made it a difficult target. “A lot of downstream target genes are regulated by SATB1. That makes for a lot of potential—and unwanted—consequences in targeting such a ‘master switch,’” he said. “A mass of targets would be affected. This might be too general of an effect.”

Young suggested one or more of the downstream proteins governed by SATB1 might make better targets against metastasis. But Kohwi-Shigematsu noted that downstream proteins have functions in normal cells and targeting them also could result in toxic side effects.

“An important future technology would be one to deliver a SATB1 inhibitor directly to breast tumors or cells,” efficiently destroying only metastatic cells, she said.

“An important future technology would be one to deliver a SATB1 inhibitor directly to breast tumors or cells.”

**— Terumi Kohwi-Shigematsu,
Lawrence Berkeley National Lab**

OncoMed's Hoey noted that a nuclear transcription factor like SATB1 would also be technically difficult to target.

"Non-enzymatic intracellular proteins have been difficult to target with small molecules," he said. "Apart from compounds targeting nuclear receptors such as peroxisome proliferation-activated receptors, the technology seems a long way from producing a drug without some kind of breakthrough."

Hoey agreed that downstream molecules regulated by SATB1 might be more accessible.

Attenuon's Mazar concurred that SATB1 was a more attractive target than CD151 for metastasis, in part because targeting SATB1 did not depend on catching metastasis before it occurred.

However, like Hoey, Mazar was not sure how to target the nuclear protein. "Is there a regulatory site on SATB1 that can be targeted with a small molecule to prevent its transcriptional activity?" he said.

"The paper goes a long way toward target validation, but I'm not sure whether SATB1 is necessarily a druggable target—many proteins aren't," Mazar said.

Pulling it all together

Zijlstra was interested in how his team's and Kohwi-Shigematsu's papers intersected at the point of cell adhesion processes, noting that SATB1 downregulated E-cadherin, a protein with a "rigid adhesion profile" that prevents normal cells from moving, he said.

"Cancer cells overcome these rigid adhesions by turning on EpCAM [epithelial cell adhesion molecule], CD44 and other more 'promiscuous' adhesion molecules," some of which the *Nature* paper shows are upregulated by SATB1, Zijlstra noted. That, he said, allows the cancer cells to make and break adhesive interactions more readily, giving them greater motility than normal cells.

Zijlstra said his team's work suggests that exogenous promotion of tumor cell adhesion—by targeting CD151 or its adhesion partners—might counter the increased motility that SATB1 induces endogenously.

Beyond that, both papers present an exciting perspective on metastasis, he said. "The papers show that tumor cells become malignant in an epigenetic manner," he said. "Because tumor cells are not genetically

"Because tumor cells are not genetically mutated, but only have changes to expression and activation, they retain their susceptibility to reversion of malignancy."

—*Andries Zijlstra, Vanderbilt University*

mutated, but only have changes to expression and activation, they retain their susceptibility to reversion of malignancy. If we can disrupt these processes with the right drugs, we can actively reverse malignancy. In other words, we can still normalize the cancer cells."

Going forward, Kohwi-Shigematsu and her team have several experiments planned.

"First we wish to determine what turns SATB1 on in breast cancer," she said, although her team has not identified any factors so far. "We are also looking for SATB1 expression in

other cancer types, to see if SATB1 could be a more generic marker for metastasis."

She said the team is already considering whether to carry out experiments correlating decreased metastasis with increased survival, as Young would like to see, but declined to disclose any details.

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Staph: the NO• have it

By Lev Osherovich, Senior Writer

A paper published in *Science* sheds light on why *Staphylococcus aureus* so successfully colonizes nasal passages and points to a new target, lactate dehydrogenase, for blocking this pathogen before it can cause systemic infection.¹ But some companies are cautious about targeting *S. aureus* in the nose and about inhibiting bacterial lactate dehydrogenase, given the potential for interference with the human version of the enzyme.

One in three people harbors quiescent staph in the nasal cavities and passages. But in immunocompromised individuals the bacteria can thrive and spread systemically, causing septicemia and toxic shock syndrome. In addition, virulent, methicillin-resistant *S. aureus* (MRSA) strains, which shrug off many widely used antibiotics, have become pervasive and thus a glaring public health threat.²

The *Science* study, from a **University of Washington** team led by Ferric Fang, professor of laboratory medicine and microbiology, solves the mystery of why staph thrives in the nose, an environment full of immune cells and antimicrobial molecules that kill most other bacteria. The key to this is an inducible bacterial lactate dehydrogenase (LDH) enzyme that helps the bug resist nitric oxide radicals (NO•), antimicrobial molecules secreted by neutrophils and macrophages.³

The paper builds on previous work describing how staph adapts to high NO• environments such as the nose.⁴ In the new report, Fang's team monitored metabolite levels in staph cultures treated with NO• in order to find the specific mechanisms that help staph survive.

"NO• is nature's antibiotic, a way for the body to arrest the growth of a number of pathogens," Fang told *SciBX*. The researchers found that NO• interferes with pyruvate dehydrogenase and pyruvate formate lyase, two bacterial enzymes that are involved in processing pyruvate during glycolysis. "These enzymes are killed off by NO•, so the bug needs a way to adapt," said Fang.

With these enzymes incapacitated, bacteria develop a redox imbalance and stop growing. However, *S. aureus* eventually resumes growth, unlike a number of related but nonpathogenic microbes that cannot adapt to NO•. Fang's team discovered that this recovery results from the NO•-induced expression of an extra copy of the *LDH* gene. LDH converts pyruvate to lactate.

According to Fang, the double dose of LDH relieves the redox imbalance caused by NO•'s damage to the pyruvate-processing machinery. In NO•-treated cells, electron transport gets backed up, leading to a shortage of NAD⁺, a coenzyme that shuttles away extra electrons generated in glycolysis. NO•-induced LDH relieves this shortage by opening up an alternative pathway that lowers pyruvate levels and restores NAD⁺ levels.

To test whether this effect was important for disease, Fang's team knocked out both copies of *LDH* in a virulent staph strain. The knock-out was sensitive to NO• in a bacterial culture assay and avirulent in

a mouse model of staph infection. However, the LDH-deficient strains could colonize and kill mice defective in NO• synthesis.

Nasal intervention

Most companies working on staph therapeutics are targeting bacteria in the blood stream or in wounds. According to Fang, however, targeting the pyruvate metabolism of staph to prevent nasal colonization could be a new strategy.

At least two biotechs—**Xoma Ltd.** and **NovaBay Pharmaceuticals Inc.**—are developing nonantibiotic bactericidal compounds to kill staph in the nose, although none of them is specifically directed at pyruvate metabolism.

Nasal eradication is Xoma's goal, according to Chief Biotechnology Officer Pat Scannon. "Clinical studies show that if you eradicate staph in the nasal epithelium, it conveys protection in hospitalized patients," Scannon told *SciBX*.

Xoma 629 is a topically administered antimicrobial that will start a Phase I trial for nasal staph infection this year. The compound is derived from a bactericidal, permeability-increasing protein.

Scannon expects pre-emptive nasal therapeutics such as Xoma 629

to be most useful in at-risk populations. "You wouldn't treat everyone in the whole world, but might use this in hospitals and dialysis settings," he said.

According to Fang, current treatment for nasal staph infection involves a course of mupirocin, a generic topical antibiotic. However, he said this strategy is increasingly futile because many patients do not stick to the multiweek regi-

men. Also, MRSA strains are resistant to mupirocin.

Like Xoma, NovaBay is also seeking to wipe out nasal staph. The company's AgaNase NVC-422 is a derivative of chlorotaurine, an oxidizing agent produced by innate immune cells, and kills bacteria through a mechanism similar to NO•.

CEO Ramin Najafi said NVC-422 broadly targets bacterial molecules rather than a single target like most antibiotics. This could make the therapeutic useful against a range of staph strains, including drug-resistant ones. Najafi thinks that NVC-422 targets such common features of all staph bacteria that drug resistance will not be an issue. "If it can kill staph, it can also kill MRSA," he said.

AgaNase NVC-422 is partnered with **Alcon Inc.** and is in Phase II trials for nasal staph and Phase I trials for catheter-associated urinary tract infections.

Other companies were more cautious about going straight for the nose. Compounds that shut down staph's pyruvate metabolism are likely to be bacteriostatic rather than bactericidal, because low levels of NAD⁺ might slow down growth but not kill the bacteria. This leaves open a wider door for resistant mutants to arise. Also, nasal staph could be hard to eliminate because of the complex topography of nasal passages.

Elizabeth Posillico, CEO of **Elusys Therapeutics Inc.**, said the Fang study was "very interesting" and that "the use of LDH to evade NO• is a very important pathway that could be used for new compounds that would reduce the virulence of staph." However, she thinks the bacterium would try to evade attempts to target LDH by developing resistance mutations.

(Continues on p. 6)

"NO• is nature's antibiotic, a way for the body to arrest the growth of a number of pathogens."

—**Ferric Fang,**
University of Washington

Putting cancer to REST

By Tim Fulmer, Staff Writer

Although previous work has shown that either increasing or decreasing the level of repressor element-1-silencing transcription factor can induce tumors, it was unclear how to go about targeting or modulating the zinc-finger DNA-binding protein. Two papers published in *Nature* separately describe the discovery of an E3 ubiquitin ligase that mediates the degradation of the transcription factor in a variety of cell types. Thus, targeting the ligase might be one strategy for modulating cellular levels of repressor element-1-silencing transcription factor and treating cancer.

Repressor element-1-silencing transcription factor (REST) has many functions: it is a transcriptional repressor in normal neuronal development,¹ an oncogene when overexpressed in neural stem cells² and a tumor suppressor in epithelial cells.³ Thus, excessively high or low levels of the protein's expression can result in cancer, depending on the cell type.

In the *Nature* papers, two independent research groups led by investigators at **Harvard Medical School** and **New York University School of Medicine** report that REST is ubiquitinated by the SCF^{β-TRCP} E3 ubiquitin ligase and targeted for proteasomal degradation in a variety of cultured cell lines. Both groups also found that prior phosphorylation of REST by an as yet unidentified kinase is required for REST ubiquitination^{4,5} (see

Figure 1, "Regulation of intracellular repressor element-1-silencing transcription factor").

The Harvard group, led by Stephen Elledge and J. Wade Harper, showed that β-TRCP-mediated degradation of REST led to the differentiation of neural stem cells. The researchers also found that in human mammary epithelial cells, overexpression of β-TRCP lowered REST levels, which in turn led to oncogenic transformation.

Elledge is a professor of genetics at Harvard Medical School and at **Brigham and Women's Hospital**. Harper is a professor of pathology at Harvard Medical School.

The NYU group, led by Michele Pagano, a professor of oncology, showed that in multiple cell types, β-TRCP-mediated degradation of REST occurred during the G2 phase of the cell cycle. Low REST levels led to activation of the transcription of Mad2, a key protein that activates the mitotic spindle checkpoint and ensures proper alignment of chromosomes prior to completion of mitosis.

The group went on to show that a degradation-resistant REST mutant caused abnormally high REST levels in G2, which in turn induced multiple mitotic defects and subsequent chromosomal instability. That mechanism, the authors said, can contribute to tumor development.

Throwing in a spanner

Now that the molecular machinery controlling REST has been elucidated, the challenge is to disrupt the process.

Harper told *SciBX* that one strategy for preventing β-TRCP-mediated degradation of REST and subsequent oncogenic transformation would be to inhibit interactions between β-TRCP and the REST phosphodegron.

(Continued from "Staph: the NOse have it," p. 5)

Moreover, Posillico is unconvinced that nasal eradication is a practical goal. "I'm not sure you would want to treat everybody just on the presumption that they've got staph in their noses," she said. "You want to wait until an individual can't handle" the staph infection before medical intervention.

However, Posillico did suggest that inhibiting nasal staph growth in combination with systemic therapeutics such as the company's ETI-211 heteropolymer antibody could be "complementary" in fighting staph infections in at-risk patients with catheters or on dialysis.

Elusys is developing ETI-211 to treat staph. The preclinical compound consists of an antibody against staph coupled to an antibody against complement receptor 1 (CR1). When the antibodies bind their targets, bacteria are brought close to red blood cells, triggering the bactericidal complement cascade.

LDH also could be a difficult target because of its central role in human metabolism.

"I think it's too early to evaluate LDH as a therapeutic target," said Joseph Patti, CSO of **Inhibitex Inc.** He said the reduction in virulence with LDH mutants was "compelling" but was concerned that compounds targeting LDH might adversely affect the human version of the enzyme, which is essential for glycolysis.

Inhibitex is developing Aurexis, an mAb against a surface protein common to most staph strains. Aurexis is in Phase II trials to treat

complicated *S. aureus* bacteremia.

Fang agreed the challenge "is to design a drug that inhibits both forms of [staph] LDH without hitting the human form. We want to characterize the structure of these proteins to see if we can develop an inhibitor."

Fang is seeking industry collaborators to explore the possibility of developing LDH therapeutics for staph. "If it looks attractive as a drug target, it would be necessary to have access to a partner in industry," he said.

Fang has not sought patents for the work in the *Science* paper.

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- Xoma Ltd.** (NASDAQ:XOMA), Emeryville, Calif.

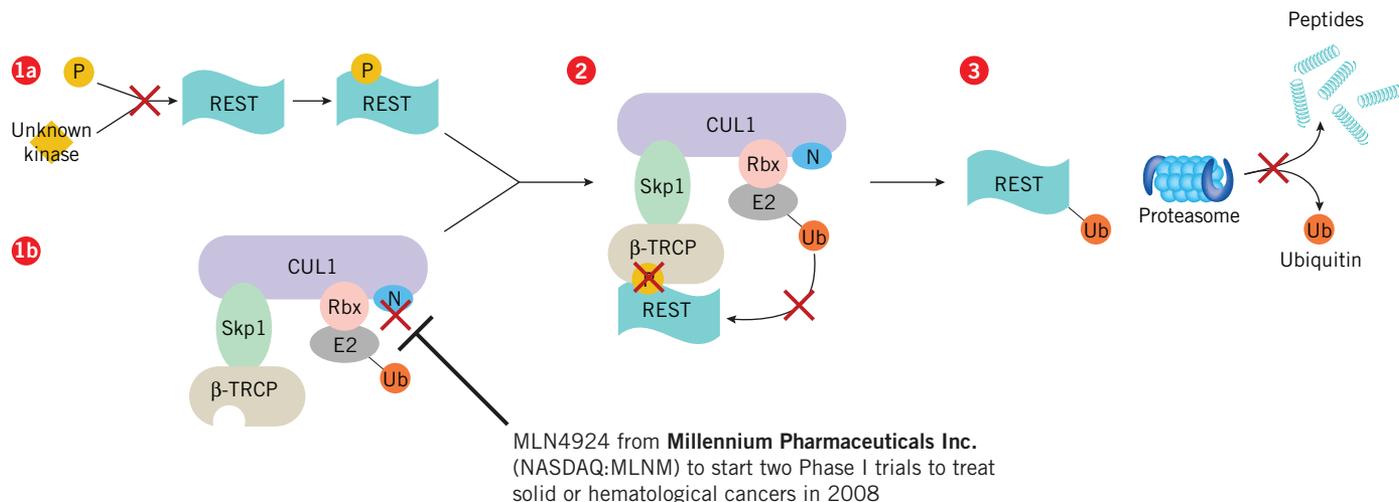


Figure 1. Regulation of intracellular repressor element-1-silencing transcription factor. Two *Nature* papers help unravel the machinery responsible for degrading repressor element-1-silencing transcription factor (REST), a potential cancer target, and reveal multiple therapeutic intervention points (marked by an X). Any therapeutic will need to be selective, however, as abnormal levels of REST can either stave off or lead to cancer depending on the type of cell. **[1a]** REST is initially phosphorylated by an as yet unidentified kinase, and blocking that phosphorylation would prevent the SCF^{β-TRCP} ligase from recognizing REST, thus saving it from downstream proteasomal degradation. **[1b]** The activated SCF^{β-TRCP} ligase is composed of the cullin 1 (Cul1) scaffold protein, to which S phase kinase-associated protein 1 (Skp1) and RING H2 finger protein (Rbx) are bound. Skp1 binds the F-box protein β-TRCP. Nedd8 (N) bound to Cul1 is required for activity of the complex; at least one biotech company, **Millennium Pharmaceuticals Inc.**, is developing a compound to block Nedd8. **[2]** β-TRCP recognizes phosphorylated REST, triggering the transfer of ubiquitin (Ub) from Ub-conjugating enzyme (E2) to REST. Blocking Ub transfer would disrupt targeting of REST to the proteasome. **[3]** Finally, ubiquitinated REST is targeted to the 20S proteasome, where it undergoes proteolysis. If that process were inhibited, cell-cycle arrest and apoptosis of cancer cells could occur.⁹

The phosphodegron is a region of the protein that comprises the phosphorylated amino acid side chains of two serine residues (S1027 and S1030) and surrounding amino acids.

“The REST phosphodegron may have a unique structural interaction with β-TRCP that could be targeted,” said Harper. Key next steps, he said, would include obtaining a crystal structure of the REST phosphodegron bound to β-TRCP, carrying out high throughput enzymatic and binding assays and developing libraries of compounds for screening.

Don Payan, EVP and president of R&D at **Rigel Pharmaceuticals Inc.**, told *SciBX* that a fundamental challenge in designing inhibitors of the β-TRCP-REST interaction is that compounds would have to disrupt “a protein-protein interaction, not a single enzyme-substrate interaction. The latter has been the modeling paradigm for programs that develop inhibitors of kinases and proteases, for example. Unfortunately this paradigm has limited usefulness in the design of E3 ligase inhibitors.”

Despite the challenge, Rigel has preclinical small molecule inhibitors that target three different E3 ligase-protein interactions: SCF-Skp2/p27 to treat cancer, Vif-BC-Cul5/APOBEC3G to prevent HIV infection and SCF-atrogin-1/calcineurin to treat muscle wasting diseases.

“Rigel’s initial strategy of using high throughput enzymatic assays to isolate E3 ligase inhibitors foundered on difficulties in identifying where

the inhibitors acted in a very complicated multisubunit complex,” said Payan. “Consequently, our more recent attempts to discover and design E3 ligase inhibitors have combined assays with computational modeling and X-ray crystal structure data, where available.”

Millennium Pharmaceuticals Inc. is taking a different approach to targeting E3 ligases: deactivating them before they have a chance to bind and ubiquitinate their substrate proteins.

“Rather than targeting the β-TRCP-REST interaction, our strategy is to go upstream from that event and target enzymes that activate the SCF^{β-TRCP} ligase in the first place,” said CSO Joe Bolen. “One such enzyme is the Nedd8-activating enzyme, which transfers the Nedd8 protein to the cullin subunit of the E3 ligase, bringing about a conformational change that activates the ligase complex. It stands to reason that inhibiting the neddylation step

would deactivate the ligase and prevent it from ubiquitinating proteins downstream.”

Added Bolen, “Designing small molecule inhibitors of an enzyme is easier than designing inhibitors of a protein-protein interaction.”

Millennium’s Nedd8-activating enzyme inhibitor MLN4924 has shown efficacy in preclinical cancer models.

“We’ve seen in tumors that MLN4924 deactivates cullin-based E3 ligases with subsequent accumulation of ligase client proteins within

“Designing small molecule inhibitors of an enzyme is easier than designing inhibitors of a protein-protein interaction.”

**—Joe Bolen,
Millennium Pharmaceuticals Inc.**

the cell and, ultimately, apoptosis,” said Bolen. “Importantly, because the Nedd8-activating enzyme is more highly expressed in cancer cells than in normal cells, inhibiting its function has a much less toxic effect on noncancer cells.”

This year, MLN4924 is slated to enter two Phase I trials to treat solid or hematological cancers.

Bolen noted that the E3 ligase described in the two *Nature* papers is a cullin-dependent ligase. “Since MLN4924 is designed to specifically target just such a ligase, there’s every reason to believe the compound could effectively modulate REST levels in some cancer cell types,” he said.

In January, Bolen added, Millennium announced a collaboration with Harper’s group at Harvard to “identify components within various ubiquitin-like post-translational modification pathways that could be targeted in cancer.”

A third approach to targeting cellular REST levels would be to inhibit the unknown kinase that phosphorylates REST. Pagano told *SciBX* that next steps in his lab include studies to identify the kinase.

Getting at the cancer

Given REST’s different functions across a number of cell types, targeting the protein to treat a particular cancer could be a delicate task.

Sadhan Majumder of the **University of Texas M.D. Anderson Cancer Center** told *SciBX* that “because REST shows oncogenic or tumor suppressor effects based on cell type, REST-specific ligase inhibitors for therapeutic purposes would probably also need to be cell- or tumor-specific.”

Majumder, a professor of cancer genetics and neuro-oncology, is focusing on the dual role REST plays as a tumor suppressor in neurogenesis and as an oncogene in medulloblastomas.^{6,7}

“Because REST shows oncogenic or tumor suppressor effects based on cell type, REST-specific ligase inhibitors for therapeutic purposes would probably also need to be cell- or tumor-specific.”

**—Sadhan Majumder, M.D.
Anderson Cancer Center**

Ian Wood, professor of biological sciences at the **University of Leeds**, is studying how REST and its co-repressors recruit chromatin-modifying enzymes that subsequently affect gene regulation.⁸ “Experiments that show tumor cells are more sensitive to loss of E3 ligase activity than normal cells—for example by using siRNA knockdown—would provide some encouragement that this could be a useful therapy,” he said.

Raymond Deshaies, professor of biology at the **California Institute of Technology**, said one approach to studying the effects of

tumor-specific targeting of E3 ligases would be to use “a mouse cancer model wherein the activity of the ligase could be regulated in some way in tumor cells—for example, by manipulating the expression of a knocked-in allele with tetracycline or using conditional knockout of a specific allele.”

Deshaies’ research focuses on the mechanisms and regulation of ubiquitin-dependent proteolysis and cell division.

Pagano is also interested in performing a mutagenesis analysis of REST in human brain tumors.

“We expect to find mutations in the phosphor-motif of REST, as already observed for β -catenin, another oncogenic substrate of β -TRCP,” he said. “These mutations would induce stabilization of REST and, consequently, chromosomal instability, thereby contributing to cell transformation.”

Indeed, Majumder and colleagues previously found that many human medulloblastomas have higher levels of both REST and the *myc* oncogene than noncancerous tissue.²

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Contact: J. Wade Harper, Harvard Medical School, Boston, Mass. e-mail: wade_harper@hms.harvard.edu
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COMPANIES AND INSTITUTIONS MENTIONED

Brigham and Women’s Hospital, Boston, Mass.
California Institute of Technology, Pasadena, Calif.
Harvard Medical School, Boston, Mass.
Millennium Pharmaceuticals Inc. (NASDAQ:MLNM), Cambridge, Mass.
New York University School of Medicine, New York, N.Y.
Rigel Pharmaceuticals Inc. (NASDAQ:RIGL), South San Francisco, Calif.
University of Leeds, Leeds, U.K.
University of Texas M.D. Anderson Cancer Center, Houston, Texas

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This week in therapeutics

THE DISTILLERY brings you this week's most essential scientific findings in therapeutics, distilled by *SciBX* editors from a weekly review of more than 400 papers in 38 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

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

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Autoimmune disease				
Systemic lupus erythematosus (SLE), arthritis and certain other autoimmune diseases	Fc- γ receptor IIb (CD32B)	A study in mice suggests that increasing the expression of CD32B on B cells might help treat autoimmune disease. In mouse models of collagen-induced arthritis (CIA), CD32B overexpression on B cells significantly reduced late-stage disease severity compared with that in control animals with normal expression of CD32B ($p < 0.05$). When CD32B was overexpressed on B cells in SLE mouse models, 85% of the transgenic mice survived at 34 weeks compared with none of the wild-type mice ($p < 0.0001$). In contrast, macrophage-specific overexpression of CD32B increased mortality in the mouse models after infection with <i>Streptococcus pneumoniae</i> . The researchers are examining the expression of CD32B in patients with various autoimmune diseases. SuppreMol GmbH's SM101 soluble form of CD32B is in preclinical testing to treat the autoimmune disease idiopathic thrombocytopenic purpura (ITP).	Not applicable	Brownlie, R. <i>et al. J. Exp. Med.</i> ; published online March 24, 2008; doi:10.1084/jem.20072565 Contact: Kenneth G.C. Smith, University of Cambridge School of Clinical Medicine, Cambridge, U.K. e-mail: kgcs2@cam.ac.uk
Cancer				
Acute lymphoblastic leukemia (ALL)	Janus kinase-1 (JAK-1)	Analyses of <i>JAK-1</i> gene mutations in DNA samples from 126 adults with ALL suggest that interrupting <i>JAK-1</i> signaling could be useful for treating adult disease. The JAK-1 protein plays an essential role in lymphocyte proliferation, survival and differentiation. Compared with patients with nonmutated <i>JAK-1</i> , those with <i>JAK-1</i> mutations had significantly lower disease-free survival (8.7 vs. 20.5 months, $p = 0.01$) and overall survival (10.6 vs. 32.5 months, $p < 0.01$). Moreover, 43% of patients with <i>JAK-1</i> mutations were resistant to induction therapy, whereas only 20% of patients with nonmutated <i>JAK-1</i> were resistant. Next steps include identifying JAK-1 inhibitors for preclinical testing.	Priority patent application filed in the U.S. for both diagnostic and therapeutic applications; licensing status undisclosed	Flex, E. <i>et al. J. Exp. Med.</i> ; published online March 24, 2008; doi:10.1084/jem.20072182 Contact: Marco Tartaglia, Istituto Superiore di Sanità, Rome, Italy e-mail: mtartaglia@iss.it
Cancer	Histone deacetylase (HDAC)	An SAR study identified small molecule ketone HDAC inhibitors that could be useful for treating cancer. One of the compounds, a 4-phenylimidazole ketone, had submicromolar antiproliferative activity in cervical, colon and kidney cell lines and micromolar activity in lung and ovarian cell lines—a profile similar to that of Zolinza vorinostat, an HDAC inhibitor from Merck & Co. Inc. that is marketed to treat cutaneous T cell lymphoma (CTCL). In a human colon HCT-116 carcinoma xenograft model, the 4-phenylimidazole ketone produced dose-dependent inhibition of tumor growth that was comparable to that from vorinostat. Next steps include developing ketone HDAC inhibitor analogs with improved activity and pharmacokinetics.	Patented; not available for licensing	Jones, P. <i>et al. J. Med. Chem.</i> ; published online March 28, 2008; doi:10.1021/jm8900079s Contact: Philip Jones, IRBM/Merck Research Laboratories, Pomezia, Italy e-mail: Philip_jones@merck.com
Cancer	Killin	An <i>in vitro</i> study suggests that targeting Killin could be part of a strategy to treat some forms of cancer. High throughput differential display identified <i>killin</i> as a p53 target gene that encodes a 20-kDa nuclear protein. Killin bound DNA with high affinity and inhibited DNA synthesis in cell culture, suggesting it might play a role in p53-mediated apoptosis of some cancer cells. Next steps include determining whether Killin activity is higher in cancer cells than in normal cells and exploring whether known tumorigenic mutations have a detectable effect on Killin function. Cleveland BioLabs Inc., Eleos Inc. and Introgen Therapeutics Inc. have compounds targeting p53 in clinical development to treat cancer.	Patent application filed; available for licensing in the U.S.	Cho, Y. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 24, 2008; doi:10.1073/pnas.0705410105 Contact: Peng Liang, Vanderbilt University Medical Center, Nashville, Tenn. e-mail: peng.liang@vanderbilt.edu

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer	Repressor element-1-silencing transcription factor (REST); Skp1-Cul1-F-box protein complex containing the F-box protein β -TRCP (SCF ^{β-TRCP})	Two separate studies in cell culture suggest that targeting the SCF ^{β-TRCP} E3 ubiquitin ligase could modulate levels of REST and help treat some forms of cancer. Depending on cell type, REST is a transcriptional repressor, an oncogene or a tumor suppressor. The Harvard group showed that in human mammary epithelial cells, SCF ^{β-TRCP} overexpression resulted in lower REST levels and oncogenic transformation. The NYU group showed that in HCT116 cell lines, abnormally high REST levels caused by a degradation-resistant REST mutant resulted in multiple mitotic defects and subsequent chromosomal instability. The Harvard group is collaborating with Millennium Pharmaceuticals Inc. to research protein homeostasis and ubiquitin-like post-translational modification pathways that potentially affect multiple cancers. Next steps for the NYU group include identifying the protein kinase that phosphorylates REST and performing a mutagenesis analysis of REST in human brain tumors. (See Putting cancer to REST, page 6.)	Patent and licensing status undisclosed	Guardavaccaro, D. <i>et al. Nature</i> ; published online March 20, 2008; doi:10.1038/nature06641 Contact: Michele Pagano, New York University School of Medicine, New York, N.Y. e-mail: michele.pagano@nyumc.org Westbrook, T. <i>et al. Nature</i> ; published online March 20, 2008; doi:10.1038/nature06780 Contact: Wade Harper, Harvard Medical School, Boston, Mass. e-mail: wade_harper@hms.harvard.edu
Cancer	Tubulin	An SAR study identified pyrano[3,2-c]pyridone and pyrano[3,2-c]quinolone compounds with antiproliferative and apoptotic activity that could be useful for treating cancer. In a human T cell leukemia cell line, two pyranoquinolones had apoptotic activity. One compound showed a dose-dependent response, and the other was potent at both low and high concentrations. In a cervical adenocarcinoma cell line, both compounds had submicromolar GI ₅₀ values. In an <i>in vitro</i> tubulin polymerization assay, both compounds completely suppressed microtubule polymerization. Next steps include studying pyranopyridone and pyranoquinolone compounds in a panel of cancer cell lines, followed by studies in mice. At least nine companies have tubulin inhibitors for multiple cancers in development stages ranging from discovery to Phase III trials.	Patent application filed; available for licensing	Magedov, I. <i>et al. J. Med. Chem.</i> ; published online March 25, 2008; doi:10.1021/jm701499n Contact: Joerg Huelsenken, Department of Chemistry, New Mexico Institute of Mining and Technology, Socorro, N.M. e-mail: akornien@nmt.edu
Chronic lymphocytic leukemia (CLL)	miR-15a; miR-16-1	A study of a leukemia cell line and primary samples from CLL patients identified a coexpressed gene signature that could be useful for diagnosing CLL. The two microRNAs, miR-15a and miR-16-1, are tumor suppressors that are absent or downregulated in CLL. A microarray analysis of gene expression in MEG-01 leukemia cells transfected with the two miRNAs revealed a signature of multiple genes that were differentially expressed compared with tumor cells that received empty vector. Many of these genes coded for proteins involved in cell cycle and antiapoptotic pathways. A subsequent analysis of primary CLLs that expressed the two miRNAs revealed a gene signature that also included downregulated antiapoptotic proteins. The researchers are testing the efficacy of i.v. administration of miR-15a and miR-16-1 in mouse models of CLL.	International patent issued covering cancer-related uses of miR-15 and miR-16 and related miRNAs; international patent application filed describing <i>BCL2</i> as a direct target of these miRNAs; both exclusively licensed to CroGen Pharmaceuticals LP	Calin, G. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 24, 2008; doi:10.1073/pnas.0800121105 Contact: Carlo M. Croce, Human Cancer Genetics Program, Department of Molecular Virology, Immunology, and Medical Genetics, Ohio State University, Columbus, Ohio e-mail: carlo.croce@osumc.edu
Liver cancer	Transmembrane 4 L six family member 5 (TM4SF5)	Studies of hepatocytes <i>in vitro</i> and in mice suggest that targeting TM4SF5 could help treat some forms of liver cancer. TM4SF5 is a transmembrane protein of the tetraspanin family that is overexpressed in human liver cancer samples. Nude mice injected with SNU449 hepatocarcinoma cells expressing TM4SF5 had higher rates of tumor formation than mice receiving hepatocarcinoma cells not expressing TM4SF5. The researchers are conducting <i>in vitro</i> and <i>in vivo</i> studies with two plant-derived small molecules that potentially target either TM4SF5 itself or TM4SF5-positive cells.	Korean and international patent applications filed covering therapeutic targeting of TM4SF5 and its tumorigenic mechanisms, as well as composition of matter on certain related chemical structures; all available for licensing	Lee, S.-A. <i>et al. J. Clin. Invest.</i> ; published online March 20, 2008; doi:10.1172/JCI33768 Contact: Jung Weon Lee, Seoul National University, Seoul, South Korea e-mail: jwl@snu.ac.kr

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Metastatic breast cancer	Id1	Studies in mice suggest that inhibiting Id1 could help treat metastatic breast and other cancers. Id1 is a helix-loop-helix transcription regulator that is commonly overexpressed in solid cancers. In mice that received transplants of mammary epithelial cells, expression of both Id1 and oncogenic Ras in the mammary epithelium increased the incidence and size of mammary tumors compared with those in mice that received Id1 or oncogenic Ras alone. However, inactivation of Id1 in established breast tumors led to widespread cellular senescence, arrest of tumor growth and regression in 40% of mice. Researchers did not disclose next steps. Peptide-conjugated antisense oligonucleotides targeting Id1 from AngioGenex Inc. are in preclinical testing to treat cancer.	Patent and licensing status undisclosed	Swarbrick, A. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 24, 2008; doi:10.1073/pnas.0801505105 Contact: Alexander Swarbrick, Garvan Institute of Medical Research, Darlinghurst, Australia e-mail: a.swarbrick@garvan.org.au
Nasopharyngeal carcinoma (NPC)	miR-29c	A study in tissue culture suggests that modulating expression of microRNA miR-29c could help treat NPC, a highly invasive cancer. Expression profiling of laser-captured microdissected NPC patient tissues identified eight miRNAs that were differentially expressed. miR-29c expression was significantly lower in cancerous tissue than in healthy tissue ($p < 0.002$). In HeLa and HepoG2 cells transfected with a miR-92c precursor, several genes encoding extracellular matrix proteins were downregulated in the presence of the miRNA. Such proteins are often associated with invasiveness and metastatic potential. Further research might examine whether miR-29c decreases metastases in NPC and other cancers.	Patent and licensing status undisclosed	Sengupta, S. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 24, 2008; doi:10.1073/pnas.0801130105 Contact: Paul Ahlquist, Institute for Molecular Virology, University of Wisconsin, Madison, Wis. e-mail: ahlquist@wisc.edu
Cardiovascular disease				
Deep vein thrombosis (DVT)	<i>Cytochrome P450, family 4, subfamily V, polypeptide 2 (CYP4V2)</i>	A genome-wide association analysis using three large case-control studies identified seven SNPs associated with risk for DVT that might offer targets to treat disease. Genotyping of about 20,000 SNPs in about 8,200 individuals identified 3 SNPs associated with DVT: rs13146272 in <i>CYP4V2</i> , rs2227589 in <i>SERPINC1</i> and rs1613662 in <i>GP6</i> ($p < 0.05$ for all). In the region near <i>CYP4V2</i> , four additional SNPs were associated with both DVT and levels of clot-promoting enzyme factor XI. Further studies could explore interactions between SNPs and identify functional consequences of the polymorphisms.	Not applicable	Bezemer, I. <i>et al. JAMA</i> ; published online March 18, 2008; doi:10.1001/jama.299.11.1306 Contact: Frits R. Rosendaal, Leiden University Medical Center, Leiden, the Netherlands e-mail: f.r.rosendaal@lumc.nl
Endocrine disease				
Obesity	Cannabinoid CB ₁ receptor	An SAR study identified a series of cannabinoid CB ₁ receptor antagonists based on an azetidinone scaffold that could be useful for treating obesity. Five of the compounds displayed >50% inhibition at 100 nM in a cannabinoid CB ₁ receptor competitive binding assay. The most potent compound had a K _i value of 53 nM and exhibited >5-fold selectivity against the cannabinoid CB ₂ receptor. Researchers did not disclose next steps. No fewer than nine companies have compounds targeting the cannabinoid CB ₁ receptor for obesity in developmental stages ranging from preclinical to marketed.	Patented by Schering-Plough Corp.; unavailable for licensing	Wang, H. <i>et al. J. Med. Chem.</i> ; published online March 26, 2008; doi:10.1021/jm701519h Contact: Hongwu Wang, Schering-Plough Research Institute, Kenilworth, N.J. e-mail: hongwu.wang@spcorp.com
Infectious disease				
HIV-1; cancer	IL-15	A study in mice suggests that IL-15 might be a useful adjuvant in vaccines that target HIV and other diseases characterized by T cell deficiencies, including cancer. One consequence of CD4 ⁺ T cell deficiency is reduced induction and maintenance of cytotoxic CD8 ⁺ T cells, which contribute to protective immunity against viral infections and cancer. In CD4 ⁺ -depleted mice, delivery of IL-15 in combination with an HIV vaccine resulted in induction and maintenance of efficacious CD8 ⁺ T cells. These T cells were also able to prevent tumor growth in the mice following the injection of fibrosarcoma tumor cells. The researchers are conducting studies of IL-15 in nonhuman primates. There are at least seven HIV vaccines in Phase I and Phase II testing.	U.S. and international patent applications filed covering recombinant vaccine viruses expressing IL-15 and methods of use; available for licensing	Oh, S.K. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 24, 2008; doi:10.1073/pnas.0801003105 Contact: Thomas A. Waldmann, Vaccine Branch and Metabolism Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Md. e-mail: tawald@helix.nih.gov

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Sepsis	CD44	A study in mice suggests that blocking interactions between the neutrophil surface receptor CD44 and hyaluronan in the liver could help treat hepatic inflammation associated with sepsis. Hyaluronan bound CD44 receptors on neutrophils and caused their recruitment to the liver. In mice, anti-CD44 antibodies or CD44 knockout lowered the number of neutrophils in the liver compared with those in the liver of untreated and wild-type control mice. Next steps include testing the safety and efficacy of anti-CD44 therapy in septic animals. Arius Research Inc.'s anti-CD44 antibody, AR001, is in preclinical development to treat cancer.	Not applicable	Kubes, P. <i>et al. J. Exp. Med.</i> ; published online March 24, 2008; doi:10.1084/jem.20071765 Contact: Paul Kubes, University of Calgary, Alberta, Canada e-mail: pkubes@ucalgary.ca
Neurology				
Alzheimer's disease (AD)	Acetylcholinesterase (AChE); butyrylcholinesterase (BChE)	An <i>in vitro</i> study characterized bis-(–)-normeptazinol derivatives as cholinesterase inhibitors that could be useful for treating AD. The most potent inhibitors had IC ₅₀ values of about 4–17 nM for AChE and BChE—an improvement over mono-(–)-meptazinol and rivastigmine by several orders of magnitude. The compounds also showed moderate inhibition of AChE-induced β -amyloid aggregation. Next steps include testing the compounds in animal models of memory impairment. Shire plc markets the opioid analgesic Meptid meptazinol to treat moderate pain. Novartis AG markets Exelon rivastigmine, a dual inhibitor of both AChE and BChE, to treat mild to moderate AD and Parkinson's disease (PD).	Patent and licensing status undisclosed	Chen, H. <i>et al. J. Med. Chem.</i> ; published online March 12, 2008; doi:10.1021/jm070154g Contact: Zhuibai Qiu, Fudan University, Shanghai, China e-mail: zbqiu@shmu.edu.cn
Huntington's disease (HD); Parkinson's disease (PD)	Huntingtin; α -synuclein	A pharmacological screen identified multiple generic drugs that could be repurposed to treat HD and PD. Five L-type Ca ²⁺ channel antagonists including verapamil and loperamide, as well as the K ⁺ _{ATP} opener minoxidil and the G _i signaling activator clonidine, promoted the clearance of α -synuclein and huntingtin aggregates by stimulating autophagy, a mechanism for digesting intracellular proteins by encapsulating them in membranous compartments. In fly and zebrafish models of HD, the generic drugs reduced huntingtin aggregation compared with no treatment. Next steps include testing the drugs or other compounds identified in the screen in mouse models of HD and PD. Verapamil is marketed to treat angina pectoris, loperamide to treat acute diarrhea, minoxidil to treat alopecia and clonidine to treat hypertension.	Patented; available for licensing through Cambridge Enterprise Ltd.	Sarkar, S. <i>et al. Nat. Chem. Biol.</i> ; published online March 23, 2008; doi:10.1038/nchembio.79 Contact: David C. Rubinsztein Department of Medical Genetics, University of Cambridge, U.K. e-mail: dcr1000@hermes.cam.ac.uk
Ischemia	Asparagine endopeptidase (AEP)	<i>In vitro</i> and <i>in vivo</i> studies suggest that inhibiting AEP could be useful for treating neurodegeneration triggered by stroke or ischemia. Under acidic conditions typical of cerebral ischemia, AEP deactivated an inhibitor of DNase, which triggered DNA damage that led to neuronal cell death. In mice, phosphoinositide 3-kinase enhancer-L (PIKE-L) bound to and protected the DNase inhibitor from degradation by AEP. That, in turn, lowered neuronal cell death. Next steps could include identifying inhibitors of AEP.	Not patented; unlicensed	Liu, Z. <i>et al. Mol. Cell.</i> ; published online March 27, 2008; doi:10.1016/j.molcel.2008.02.017 Contact: Keqiang Ye, Emory University School of Medicine, Atlanta, Ga. e-mail: kye@emory.edu

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This week in techniques

THE DISTILLERY brings you this week's most essential scientific findings in techniques, distilled by *SciBX* editors from a weekly review of more than 400 papers in 38 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

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

Approach	Summary	Licensing status	Publication and contact information
<i>In vivo</i> delivery of microRNA antagonists	A study in mice and primates suggests that unconjugated locked nucleic acid (LNA)-modified oligonucleotides could be superior to unmodified oligonucleotides for antagonizing miRNAs and treating disease. In hypercholesterolemic mice, twice-weekly administration of LNA-modified anti-miR-122 reduced total plasma cholesterol compared with levels in mice that received LNA-mismatch or saline controls. In African green monkeys, thrice-weekly administration of LNA-modified anti-miR-122 lowered total plasma cholesterol compared with levels in monkeys that received saline control. miR-122 is expressed in the liver and is implicated in cholesterol metabolism, lipid metabolism and HCV replication. Next steps include optimizing the dosing regimen and studying long-term safety in animals before testing the miRNA antagonists in humans. Santaris Pharma A/S plans to start a Phase I safety and pharmacokinetics trial of SPC3649, an LNA-anti-miR targeting miR-122 to treat HCV, in healthy volunteers this year.	miRNA silencing method patented by Santaris; GlaxoSmithKline plc has an option to license SPC3649 from Santaris under a 2007 agreement; method unavailable for licensing	Elmen, J. <i>et al. Nature</i> ; published online March 27, 2008; doi:10.1038/nature06783 Contact: Sakari Kauppinen, Santaris Pharma, Horsholm, Denmark e-mail: sk@santaris.com
Search algorithm for identifying efficacious drug combinations	A closed-loop optimization approach using a stochastic search algorithm could be a useful strategy for identifying drug combinations that modify cellular activities more effectively and with lower doses than a single drug. Fewer than 15 search iterations of 100,000 possible drug combinations identified cocktails of interferons (IFNs) and antivirals that prevented vesicular stomatitis virus (VSV) infection of fibroblasts. The approach also identified a combination of cytokines that maximized activity of NF-κB. Next steps include preclinical testing of the identified drug combinations.	Patent application filed; licensing status undisclosed	Wong, P. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 17, 2008; doi:10.1073/pnas.0800823105 Contact: Pak Kin Wong, University of Arizona, Tucson, Ariz. e-mail: pak@email.arizona.edu
Virtual screening for GPCR antagonists	A four-step virtual screening method could be superior to current GPCR virtual screens for identifying therapeutic leads to treat multiple diseases. Unlike previous GPCR screens, the new screen includes conformational flexibility and multiple transmembrane interactions of GPCRs. Using a model of the thyrotropin-releasing hormone complexed with its GPCR (TRH-R), a virtual screen of more than 1 million commercially available compounds identified antagonists with specificity for TRH-R1 over the almost identical TRH-R2. Further studies are necessary to optimize the compounds and identify more clinically relevant GPCR ligands. Compugen Ltd. has a virtual ligand discovery technology to identify GPCR agonists.	Neither the method nor the antagonists have been patented; unavailable for licensing	Engel, S. <i>et al. J. Am. Chem. Soc.</i> ; published online March 24, 2008; doi:10.1021/ja0776201 Contact: Marvin C. Gershengorn, National Institutes of Health, Bethesda, Md. e-mail: marving@intra.niddk.nih.gov

INDEXES

Company and institution index

A		C		F		Italian Foundation for Cancer Research Institute for Molecular Oncology	1
Alcon Inc.	5	California Institute of Technology	8	Fox Chase Cancer Center	3		
AngioGenex Inc.	11	Cambridge Enterprise Ltd.	12	G		L	
Arius Research Inc.	2,12	Cleveland BioLabs Inc.	9	GlaxoSmithKline plc	3,13	Lawrence Berkeley National Laboratory	3
Attenuon LLC	2	Compugen Ltd.	13	H		M	
B		CroGen		Harvard Medical School	6	Merck & Co. Inc.	9
Brigham and Women's Hospital	6	Pharmaceuticals LP	10	I		Millennium Pharmaceuticals Inc.	7,10
		E		Inhibitex Inc.	6		
		Eleos Inc.	9	Innovascreen Inc.	1		
		Elusys Therapeutics Inc.	5	Introgen Therapeutics Inc.	9		

N		BChE	12	K		S	
New York University School of Medicine	6	<i>BCL2</i>	10	Killin	9	S phase kinase-associated protein 1	7
NovaBay		Bis(-)-normeptazinol	12	L		SATB1	1
Pharmaceuticals Inc.	5	Butyrylcholinesterase	12	Lactate	5	SCF-Skp2/p27	7
Novartis AG	12	C		Lactate dehydrogenase	5	SCF-atrogin-1/calcineurin	7
O		Cadherin-11	3	LDH	5	SCF ^{β-TRCP}	6,10
OncoMed		Cadherin-5	3	Loperamide	12	SCF ^{β-TRCP} E3 ubiquitin ligase	10
Pharmaceuticals Inc.	3	Cannabinoid CB ₁ receptor	11	M		<i>SERPINC1</i>	11
R		CD144	3	Mad2	6	Skp1	7
Rigel Pharmaceuticals Inc.	7	CD151	1	Meptid	12	Skp1-Cul1-F-box protein complex containing the F-box protein β-TRCP	10
S		CD32B	9	Minoxidil	12	SM101	9
Santaris Pharma A/S	13	CD44	2,12	miR-122	13	SPC3649	13
Schering-Plough Corp.	11	Chlorotaurine	5	miR-15a	10	Special AT-rich sequence binding protein 1	1
Scripps Research Institute	1	Clonidine	12	miR-16-1	10	T	
Shire plc	12	Complement receptor 1	6	miR-29c	11	TACSTD2	2
State University of New York at Stony Brook	1	CR1	6	MLN4924	7	Tetraspanin	1
SuppreMol GmbH	9	Cul1	7	Mono(-)-meptazinol	12	Tetraspanin 24	1
U		Cullin	7	Mupirocin	5	Thyrotropin-releasing hormone	13
University of Leeds	8	Cullin 1	7	N		TM4SF5	10
University of Texas M.D. Anderson Cancer Center	8	CYP4V2	11	N-cadherin	3	Transmembrane 4 L six family member 5	10
University of Washington	5	<i>Cytochrome P450, family 4, subfamily V, polypeptide 2</i>	11	NAD ⁺	5	TRH-R1	13
V		E		Nedd8	7	TRH-R2	13
Vanderbilt University	2	E-cadherin	3	Nedd8-activating enzyme	7	TROP2	2
X		E3 ubiquitin ligase	6	NF-κB	13	Tubulin	10
Xoma Ltd.	5	EpCAM	4	Nitric oxide radical	5	Tumor-associated calcium signal transducer 2	2
.....		Epithelial cell adhesion molecule	4	NO•	5	U	
Target and compound index		ETI-211	6	NVC-422	5	Ubiquitin	8,10
4-Phenylimidazole ketone	9	Exelon	12	O		V	
A		F		OB-cadherin	3	VE-cadherin	3
α-synuclein	12	Fc-γ receptor IIb	9	OMP-21M18	3	Verapamil	12
Acetylcholinesterase	12	G		p53	9	Vif-BC-Cul5/APOBEC3G	7
AChE	12	<i>GP6</i>	11	Peroxisome proliferation-activated receptor	4	VLA-3	2
AEP	12	GPCR	13	Phosphoinositide 3-kinase enhancer-L	12	Vorinostat	9
AgaNase	5	H		PIKE-L	12	X	
AR001	2,12	HDAC	9	Pyrano[3,2-c]pyridone	10	Xoma 629	5
AR002	2	Histone deacetylase	9	Pyrano[3,2-c]quinolone	10	Z	
Asparagine endopeptidase	12	Huntingtin	12	Pyruvate	5	Zolinza	9
ATN-161	2	Hyaluronan	12	Pyruvate dehydrogenase	5		
Aurexis	6	I		Pyruvate formate lyase	5		
Azetidinone	11	Id1	11	R			
B		IL-15	11	Ras	11		
β-amyloid	12	Integrin	1	Rbx	7		
β-catenin	8	Integrin α ₃ β ₁	2	Repressor element-1-silencing transcription factor	6,10		
β-TRCP	6	Integrin α ₅ β ₁	2	REST	6,10		
		Integrin α ₆ β ₁	2	RING H2 finger protein	7		
		Integrin α _v β ₃	2	Rivastigmine	12		
		J					
		JAK-1	9				
		Janus kinase-1	9				