

**THIS WEEK****ANALYSIS****COVER STORY****1 Merck's Rosetta stone**

A series of studies integrating genomics and bioinformatics methods paints the clearest picture yet of how the many facets of genomic organization and regulation fit together to influence disease. The approach, developed by Merck's Rosetta Inpharmatics unit, is shaping the pharma company's drug discovery efforts.

**TARGETS & MECHANISMS****5 Cancer's long-distance plan**

Cancer researchers have long known that cells in the tumor microenvironment support cancer growth. But MIT researchers and colleagues now suggest that cells quite distant from the tumor may play an equally important role in instigating tumor proliferation; they also suggest that the systemic endocrine factors mediating such long-distance interactions could provide new targets for cancer therapy.

**7 DegrADing dementia**

Separate research teams suggest that stimulating protease-modulating factors in the brain may boost  $\beta$ -amyloid degradation and thus slow progression of Alzheimer's disease. This may allow the repurposing of existing compounds targeting these factors—if challenges of dosage, specificity and brain permeability can be overcome.

**10 The path of Iressa resistance**

Researchers writing in *JCI* have provided a mechanistic rationale for how combining growth factor receptor inhibitors may help overcome resistance to Iressa gefitinib in cancer. Companies working in the space differ on whether the mechanism will apply to resistance to other EGFR inhibitors such as Tarceva erlotinib.

**THE DISTILLERY****12 This week in therapeutics**

Treating graft-versus-host disease with HDAC inhibitors; using ERCC5 as a response marker for cancer therapy; preventing colon cancer by immunizing with GUCY2C; and more...

**16 This week in techniques**

Synthetic attenuated virus engineering; chemically mediated somatic cell reprogramming; aptamer-mediated biomarker discovery; and more...

**INDEXES****18 Company and institution index****18 Target and compound index****Merck's Rosetta stone**

By Lev Osherovich, Senior Writer

Four papers published in *Nature Genetics*, *Nature* and *Public Library of Sciences Biology* provide a compelling proof of concept for the use of integrative genomic analysis to drive the discovery of genes underlying complex diseases such as obesity and other metabolic disorders. Genomics companies told *SciBX* that the approach described in these papers had been considered technically unfeasible, but now its application could significantly accelerate marker and target discovery.

Indeed, the integrative approach reported in the papers, which was developed by **Merck & Co. Inc.** subsidiary **Rosetta Inpharmatics LLC**, is already shaping the pharma company's drug discovery efforts.

Genome mining for new therapeutic targets often focuses either on genotype mapping or gene expression analysis. Due to the volume and complexity of information in genome-wide data sets, researchers have had only limited success at integrating these different levels of genomic information.

By contrast, the new studies paint the clearest picture yet of how the many facets of genomic structure and regulation fit together to influence disease.<sup>1</sup>

Three of the studies were from combined industry and academic teams led by Eric Schadt, executive scientific director of genetics at Rosetta, whereas the fourth study was co-led by Kari Stefansson, CEO of **deCODE genetics Inc.**

All four studies integrate genotype and gene expression analysis with additional data, including transcription factor binding sites and protein-protein interactions as well as physiological measurements. Three studies used data from yeast, mice and human tissue samples. The study coauthored with Stefansson analyzed human clinical data.

**Master genes**

The main method used in the four studies was expression quantitative trait locus (eQTL) mapping, which identifies chromosomal regions that regulate the expression of genes associated with disease.<sup>2</sup>

Conventional QTL mapping identifies SNPs that correlate with elevated disease risk, pointing to hereditary factors that influence disease, but it does not shed light on how these factors interact with each other in disease.

In contrast, eQTL analysis aims to map markers near master regulator genes that control the expression of large groups of other genes involved in disease. eQTL mapping can identify gene networks and their master switches even in healthy subjects, making it particularly



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useful for studying complex and environmentally influenced diseases like metabolic syndrome.

eQTL mapping gathers gene expression data using mRNA microarrays and then looks for correlations between individual SNPs and genome-wide patterns of gene expression indicative of disease.

According to Schadt, eQTL overcomes the limits of conventional gene mapping by uncovering pivotal genes in diseases that are not purely heritable, but are influenced by multiple genes. Although QTL studies can identify risk loci, eQTL can unravel the relationships between them.

“Complex traits such as common human diseases or drug response result from many genes interacting in complex ways,” Schadt told *SciBX*. “You have to look beyond DNA variation into a space that incorporates both DNA variation and gene activity.”

Like conventional QTL mapping, eQTL points to fairly broad chromosomal intervals but doesn't directly identify candidate genes. To find the lynchpin genes, Schadt's team gathered additional experimental, clinical and genomic data and then combined the results to generate theoretical models of genetic networks that best explained the data.

Schadt told *SciBX* that Merck's acquisition of Rosetta in 2001 allowed the company to build and staff a customized computing facility to handle the massive amount of data involved in the studies.

“Getting and managing the data is technically challenging,” he said. “The number of combinations you have to consider is large. We're getting into terabytes of data” per single experiment, he noted.

**Yeast and mouse proof**

As a proof of principle, Schadt's team deployed the eQTL technique in yeast and in mice. In these organisms, the genetic variation needed to create an eQTL map can be generated by crossing unrelated strains and analyzing their offspring. The yeast study appeared in *Nature Genetics*,<sup>3</sup> whereas the mouse study was published in *Nature*.<sup>4</sup>

In the yeast study, Schadt's team used pre-existing DNA sequence and gene expression data from a panel of genetically related yeast strains to identify ‘hot spots’—areas of the genome with particularly strong eQTL signals.

To find the master genes hidden in the hot spots, the team computationally compared the eQTL data with published information about protein-protein interactions and transcription factor binding sites. This analysis yielded a list of likely master regulator genes involved in yeast metabolism.

Indeed, strains lacking these critical regulators showed changes in the expression of the target genes predicted by the analysis.

Schadt's team also used eQTL in mice to identify genes that influence obesity and other metabolic traits. Focusing on a portion of chromosome 1 that previously had been implicated in metabolic disease, the researchers conducted an eQTL scan of adipose tissue using both gene expression data and measurements of weight, fat mass and cholesterol levels.

The most likely suspects turned out to be a network of genes expressed in macrophages, a type of innate immune cell. Previous studies had suggested that macrophage infiltration of adipose tissue was linked with obesity, but the mechanisms of this process were unknown.<sup>5</sup>

Schadt's team termed the group of these coordinately regulated genes the macrophage-expressed metabolic network (MEMN).

To test their predictions, the team disrupted several of the MEMN genes and measured the effect on obesity-associated traits. Mice lacking one copy of *lipoprotein lipase (Lpl)*—one of the MEMN genes—had 22% higher fat-mass-to-lean-mass ratio than wild-type littermates. Likewise, overexpression of another protein in the network,  $\beta$ -lactamase, made mice 20% fatter than wild-type controls.

The strongest master regulator eQTL signal in the mouse study came from another MEMN gene, which encodes a newly discovered protein phosphatase called protein phosphatase 1 (formerly 2C)-like (Ppm1L; Ppm1-like). Schadt's team found that

*Ppm1L* knockouts displayed many characteristics of metabolic syndrome, including faster weight gain, higher adult weight and higher fat mass than wild-type controls. *Ppm1L* knockouts had hyperinsulinemia, high glucose tolerance and higher blood pressure than wild-type controls, all of which are characteristics of human metabolic syndrome.

Ppm1L thus could be a target for drug development to treat metabolic syndrome. Alternatively, *Ppm1L* knockout could be a new mouse model for the disorder.

### From mice to men

Concurrent with the mouse study, Schadt's team collaborated with deCODE to tease out human metabolic syndrome genes. The work, reported in the second *Nature* article, involved eQTL analysis of tissue samples from hundreds of Icelanders.<sup>6</sup>

deCODE uses Iceland's complete genetic and medical information to hunt for disease-associated genes and to develop diagnostics.

The Merck and deCODE team analyzed the expression of 84% of the human genome in adipose tissue and blood from 1,675 Icelanders, integrating these data with genotype analysis of 1,732 microsatellite markers, which are repetitive noncoding DNA sequences often used in gene-mapping studies.

An additional level of precision came from an analysis of 317,503 SNPs in a subset of the subjects. As the hereditary relationships of all Icelanders are known, eQTL signals were also correlated to patterns of inheritance, thus improving the method's accuracy.

The team also analyzed clinical data such as body mass index (BMI), percentage body fat and waist-to-hip ratio from each individual. These metrics have previously been used to describe obesity and to predict susceptibility to metabolic syndrome.<sup>7</sup>

By computationally merging the clinical, genotypic and eQTL data, the team homed in on the genes most likely to underlie obesity. In fact, many candidate genes belonged to the MEMN, just as in mice. Variation in expression of most MEMN genes correlated with BMI variation, although the correlation with percent body fat was not as strong.

The *Nature* study did not reveal whether human homologs of the three mouse genes described in Schadt's *Nature* paper were also relevant in human obesity. However, according to deCODE's website, one of three genes identified in the now completed collaboration is

the target of a preclinical compound being developed by Merck. The pharma company would not disclose the target or development status of the compound.

deCODE and Merck finished joint work exploring the human genome for obesity targets in 2005.

Finally, a study of eQTLs in human livers by Schadt and collaborators at five academic institutions, published in *PLoS Biology*, identified *Sortilin 1 (SORT1)* and *Cadherin EGF LAG seven-pass G-type receptor 2 (CELSR2)* as candidate susceptibility genes influencing coronary artery disease and plasma low-density lipoprotein (LDL) cholesterol levels.<sup>8</sup>

Schadt said the method could lead to faster transition from gene discovery to therapeutic development. He noted that the

Rosetta team's gene discovery methods work hand in hand with the human gene-targeting technologies being developed by another Merck subsidiary, **siRNA Therapeutics Inc.**

"Once you have a network that defines a disease state, you can go into a human experimental study and hit that network with different [short interfering] RNAs," he said. "Merck's siRNA technology lets us start thinking about multiple nodes at once and going into experimental clinical studies."

### Other perspectives

Genomics companies told *SciBX* that Schadt's studies are proof of principle of methods previously thought to be technically unfeasible, and that they will significantly accelerate marker and target discovery.

"Genome-wide association studies with whole-genome SNP platforms and gene expression are not new," said Tod Klingler, VP of information sciences at molecular diagnostics company **XDx Inc.** "The innovation is the series of informatics and data analysis."

He added: "Network modeling is really brand new. These are significant experiments."

In genomics studies of this size, said Klingler, "you often have to worry about false discovery rates." However, he said, Schadt's method of grouping genes in networks and pathways greatly improves the chances of "finding the signal in the noise."

XDx markets AlloMap, a molecular expression screening service for diagnosing risk of acute cellular rejection after cardiac transplant.

"Merck's approach could be used to better organize the gene expression results that we're getting," said Russ Dietrich, XDx's director of molecular immunology.

Gualberto Ruaño, president and CEO of **Genomas Inc.**, told *SciBX* that Schadt's integration of clinical data into the eQTL analysis makes the work highly innovative.

"Technically, it's a tour de force to do so many biopsies of so many individuals," he said. "Looking at gene expression is a step in the right direction."

Genomas uses genomic marker analysis together with clinical measurements to help doctors manage risks of side effects in cardiac and neuropsychiatric therapy, said Ruaño.

Both Klingler and Ruaño agreed the most immediate application of Schadt's methods is to discover diagnostic markers and to understand the organization of gene networks influenced by disease.

**"Network modeling is really brand new. These are significant experiments."**

— **Tod Klingler, XDx Inc.**

However, Rúaño cautioned that the genes identified as relevant to a disease process will likely be relevant to other processes involved in normal physiology as well. “These genes are too far upstream” to typically be useful targets, he said.

According to Merck spokesperson Caroline Lappetito, the company “has filed patent applications on some of the broader concepts developed by Schadt but has chosen not to protect most of the more detailed methods and algorithms being developed by his group.”

“Merck will seek to protect novel targets and biomarkers identified as a result of using the unique methods developed by Schadt’s group,” she added.

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# Cancer's long-distance plan

By Tim Fulmer, Senior Writer

Researchers at the **Massachusetts Institute of Technology** have uncovered a new role for the endocrine factor osteopontin as a 'long-distance' modulator of tumor growth. Researchers polled by *SciBX* felt that the findings not only opened new avenues for the design of tumor-targeting strategies but also had important implications for current surgical and therapeutic procedures aimed at tumor shrinkage.

Cancer researchers have long appreciated that cells in the immediate vicinity of tumors—the tumor microenvironment—support tumor growth and are therefore potential therapeutic targets.<sup>1,2</sup> However, the paper published in *Cell* by Robert Weinberg, professor of biology at MIT, and colleagues now suggests a potentially equally important tumor-supportive role for cells further away from a tumor.

Osteopontin (secreted phosphoprotein 1), the systemic endocrine factor that potentially mediates such long-distance interactions, could be a new cancer target. Osteopontin had previously been shown to be overexpressed in tumors and had been suggested to be involved in metastasis, but a causal connection had not yet been established.<sup>3</sup>

Weinberg and colleagues developed a mouse model for identifying systemic mechanisms that could be targeted to prevent or slow progression of solid tumors.<sup>4</sup> They implanted two different human mammary epithelial tumor cell lines on opposite sides of the same nude mouse. One of these cell lines, the 'instigator,' resembled aggressively invasive adenocarcinomas often found in breast cancer. The other cell line, the 'responder,' mimicked much slower growing indolent tumors.

Initial experiments revealed that responder cells implanted opposite instigator cells had higher growth kinetics and tumor mass than responder cells implanted opposite Matrigel control matrix.

Moreover, conditioned media from instigator tumor cells did not alter the growth of responding cells *in vitro*, suggesting that growth factors derived from the instigator tumor were not directly responsible for increasing the growth of responder tumors.

The authors speculated that "the instigating tumors might mobilize stromal cell precursors from the bone marrow into circulation, thereby making them available for recruitment by responding tumors."

A second set of experiments in the same models revealed that responder tumors opposite instigator tumors incorporated significantly more host bone marrow cells into their stroma than did responders opposite Matrigel control matrix ( $p=0.039$ ).

A plasma analysis of mice bearing only instigator tumors revealed that circulating levels of the glycoprotein osteopontin were threefold higher than those seen in mice with only responder tumors. Circulating levels of 80 other cytokines were not significantly altered between the two groups.

The researchers thus suggested that osteopontin released by instigator tumors might be responsible for recruiting bone marrow cells to responder tumor cells. In the same mouse models, they found that osteopontin-deficient instigator tumors induced less bone marrow cell recruitment and tumor growth in responder tumors than was

seen in responder tumors opposite osteopontin-expressing instigator tumors.

In conclusion, the authors proposed a "Model of Systemic Instigation," whereby primary tumors secrete soluble osteopontin, which stepwise leads to activation of bone marrow cells, release of stromal precursor cells into systemic circulation and instigation of outgrowth at previously indolent distal tumors.

Weinberg told *SciBX* that his lab now plans to use the same mouse models to study the effects of antiosteopontin antibodies on tumor instigation, as well as to identify additional systemic factors that potentially cooperate with osteopontin to promote outgrowth of distal, secondary tumors.

"The *Cell* paper illustrates the critical importance of *in vivo* context to our understanding of cancer. In particular, the researchers have shown that primary tumors can co-opt host systemic factors to promote the growth of tumors that might otherwise remain latent. Thus, at least in this case, host context provides the necessary means to cancer progression," said Murray Robinson, SVP of oncology for **Aveo Pharmaceuticals Inc.**

## New role for old player

Researchers told *SciBX* that the *Cell* paper had important implications for tumor-targeting strategies. Moreover, they said the findings throw some doubt on surgical and therapeutic procedures aimed at tumor shrinkage.

In healthy individuals, the glycoprotein osteopontin is produced by multiple cell types, including epithelial cells, bone cells, macrophages and lymphocytes. The protein interacts with a variety of cell-surface receptors, such as integrins and CD44, to stimulate cell adhesion, migration and intracellular signaling.<sup>5</sup>

At the same time, elevated osteopontin has correlated with the metastatic potential of multiple cancer cell lines.<sup>6,7</sup> Moreover, abnormally elevated plasma levels of osteopontin have been observed in some patients with metastatic renal cell carcinoma and breast cancer.<sup>8,9</sup>

"The *Cell* paper is an impressive piece of work because it does more than suggest a correlative relation between osteopontin levels and cancer," said Dennis France, VP of molecular oncology at **ArQule Inc.** "Indeed, Weinberg and colleagues have perhaps uncovered a causal role for osteopontin in modulating tumor-stroma interactions."

ArQule's lead compound is ARQ 197, a small molecule inhibitor of c-Met receptor tyrosine kinase in Phase II trials to treat Microphthalmia Transcription Factor (MiT) tumors and Tarceva erlotinib-resistant non-small cell lung cancer (NSCLC). Tarceva, a small molecule inhibitor of epidermal growth factor receptor, is marketed by **Genentech Inc., OSI Pharmaceuticals Inc. and Roche.**

The *Cell* paper shows for the first time that osteopontin promotes tumor outgrowth by influencing the contribution of bone marrow-derived cells to the tumor stroma, noted Natalie Direkze, a researcher in the histopathology unit of the **Cancer Research UK, London Research Institute.** Direkze and colleagues have shown that bone marrow cells contribute to fibroblast populations in the tumor stroma of a mouse model of pancreatic cancer.<sup>10</sup>

According to David Young, president and CEO of **Arius Research Inc.,** "the paper's model, whereby primary tumors enhance the growth of secondary tumors, stands in contrast to much of our current

understanding of how metastasis works. Multiple studies have established that resecting primary tumors can lead to metastasis because intact primary tumors release factors that suppress tumor outgrowth at secondary sites. The *Cell* paper now suggests that primary tumors can also release factors that drive growth of secondary tumors.”

Young added: “Another somewhat counterintuitive implication of the paper is that a comparatively small instigating tumor can perhaps drive the growth of a larger, previously indolent secondary tumor. Thus, surgically removing the larger tumor may, at least in this case, have little curative effect—which is certainly surprising.”

Arius plans to start a Phase I cancer trial of its AR001 anti-CD44 antibody at year end.

## Inhibiting a generalist

Andrew Mazar, CSO of **Attenuon LLC**, said a straightforward approach to antagonizing osteopontin would be an mAb.

“I would try to come up with a panel of antibodies that targeted the various domains and interactions of osteopontin and then use these to figure out which interaction was most important to the antitumor activity of osteopontin,” he said. “The high degree of homology between mouse and human osteopontin should make this doable in mice.”

Nevertheless, Mazar and other researchers polled by *SciBX* suggested that inhibiting osteopontin in cancer could bump into the usual challenges associated with targeting a molecule that interacts with a variety of receptors and cell types.

Key issues include determining which of these interactions are important for tumor-promoting activity and whether blocking a single interaction or multiple interactions is necessary to show clinically meaningful activity, Mazar said.

Attenuon’s ATN-224, a small molecule inhibitor of superoxide dismutase 1 (SOD1) that disrupts multiple kinase signaling pathways, is in separate Phase II trials to treat multiple myeloma (MM), advanced melanoma, prostate cancer and breast cancer.

Young noted that any therapeutic strategy must take into account that tumors have alternative means of replenishing their stroma.

“An antiosteopontin antibody or a soluble osteopontin decoy receptor could both be effective at blocking recruitment of bone marrow-derived cells to tumor stroma,” he said. “However, these strategies may have little or no effect on stromal precursors that are in circulation or on stromal cells that are already proliferating within the tumor microenvironment.”

Moreover, Aveo’s Robinson noted that “tumors have considerable genetic and epigenetic heterogeneity. This, in turn, makes it unlikely that a single therapeutic agent will be equally efficacious against all tumors, even of the same tissue type. Therefore, in the case of osteopontin antagonists, we ought to look for antitumor efficacy across a whole population of, say, breast cancer tumors rather than just the two types used in the paper.”

Robinson added: “Such a population would better represent the heterogeneity that characterizes breast cancer in patients and also help differentiate breast cancers where osteopontin is perhaps worth targeting from those types that may be more resistant to osteopontin [targeting].”

Aveo’s AV-951, a small molecule inhibitor of VEGF receptor 1, VEGF receptor 2 and VEGF receptor 3, is in Phase II trials to treat

metastatic renal cell carcinoma (RCC).

## Modifying the model

While the therapeutic issues involved in inhibiting osteopontin are sorted out, the models in the *Cell* paper also could be modified to investigate osteopontin–bone marrow and bone marrow–tumor stroma interactions.

France pointed out that the models in the paper were variations on standard human tumor xenograft models and therefore were immunocompromised. “Such models likely don’t have a fully responsive bone marrow that supports production of all hematopoietic cells—which means these models may not be ideal for studying osteopontin–bone marrow interactions,” he said. “Consequently, I would like to see some of this work repeated in a nonimmunocompromised syngeneic rodent model.”

Young, however, told *SciBX* that engineering knockouts into the same immunocompromised models could provide insights into osteopontin–bone marrow interactions.

“First of all, knockout of osteopontin or the osteopontin receptor in bone marrow could reveal whether other unidentified factors also mobilize stromal precursors to secondary tumors,” he said. “Secondly, knockout of hematopoietic precursor populations within the bone marrow could help elucidate the relative importance of these subpopulations for recruitment to distal tumor stroma.”

A third option, according to Peter Lassota, divisional VP of imaging biology and oncology at **Caliper Life Sciences Inc.**, would be to engineer the models to express tumor-specific or bone marrow-specific bioluminescent markers. This would allow for noninvasive monitoring of tumor growth in response to osteopontin knockout or osteopontin overexpression.

Caliper markets a range of transgenic mouse models that can be engineered to express the *luciferase* gene in a tissue-specific manner.

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# DegrADing dementia

By Lev Osherovich, Senior Writer

Two papers, one published in *Neuron* and the other in *Proceedings of the National Academy of Sciences*, suggest, respectively, that stimulating apolipoprotein E or inhibiting plasminogen activator inhibitor-1, two protease-modulating factors in the brain, may boost  $\beta$ -amyloid degradation and thus slow down progression of Alzheimer's disease.<sup>1,2</sup> Existing compounds that modulate these targets could be repurposed for the disease if challenges of dosage, specificity and brain permeability can be overcome.

Efforts to treat Alzheimer's disease (AD) have largely focused on blocking the production of  $\beta$ -amyloid (A $\beta$ ) fragments or preventing their accumulation into toxic aggregates outside neurons. The *Neuron* and *PNAS* papers show that the same goal could be achieved by stimulating extracellular proteases to boost the degradation of A $\beta$ .

Extracellular and microglial proteases such as neprilysin (NEP), insulin-degrading enzyme (IDE) and plasmin have previously been linked to A $\beta$  clearance but were considered poor direct targets because of the difficulty in enhancing their activity.<sup>3</sup> The new studies describe roundabout ways to boost protease activity: by stimulating apolipoprotein E (ApoE), a protease-assisting lipoprotein cofactor genetically implicated in AD progression, and by inactivating plasminogen activator inhibitor-1 (PAI-1), an inhibitor of a brain protease cascade.

The proteases in the two studies are controlled by several other proteins that have already been explored in cardiovascular and endocrine indications.

David Lomas, professor of medicine at the **Cambridge Institute for Medical Research**, noted that many compounds that block A $\beta$  production or aggregation in mice have not panned out in humans, but he told *SciBX* the idea of stimulating degradation is clever and warrants testing in clinical trials. Indeed, **Wyeth** has started a clinical trial of PAZ-417, a new compound described in one of the studies.

## Chew it up

The study published in *Neuron* was led by Gary Landreth, professor of neurosciences at **Case Western Reserve University**. His team used cell culture and *in vivo* techniques to uncover how ApoE, a lipoprotein involved in cholesterol metabolism, helps brain-specific immune cells called microglia take up and degrade A $\beta$ .

The study suggests that ApoE acts as a chaperone to facilitate proteolysis of A $\beta$  by both microglial and extracellular proteases.

Ordinarily, A $\beta$  accumulates into small, highly toxic oligomers and larger amyloid fibrils. Microglia have previously been shown to block this process by internalizing both soluble and fibrillar forms of A $\beta$ , as well as secreting NEP and IDE to degrade extracellular A $\beta$  that escapes ingestion.<sup>4</sup>

However, excessive microglial activity near A $\beta$  plaques may promote inflammation, thus exacerbating neurodegeneration. Researchers are still sorting out whether microglia ultimately play a beneficial or

harmful role in the progression of disease.<sup>5</sup>

Landreth's team found that adding ApoE to cultured microglia boosted their ability to internalize and degrade A $\beta$  compared with that of untreated controls. As a bonus, ApoE also enhanced the extracellular degradation of A $\beta$  by IDE compared with that of ApoE-deficient controls.

"We found that you can increase the clearance of A $\beta$  by increasing the amount of ApoE," said Landreth.

The study offers a new hypothesis about how a common variant of the *ApoE* gene raises AD risk. Landreth's team found that ApoE4, an AD-associated variant of ApoE, did not work as well as wild-type ApoE in stimulating microglial A $\beta$  degradation.

## More lipid

The key difference between normal ApoE and AD-associated ApoE4 turned out to be the amount of lipids in the particle. Compared with other ApoE variants, ApoE4 particles are smaller and have fewer lipid molecules, according to Landreth. In mutant mice unable to add lipids to ApoE, the lipid-free particles could not facilitate A $\beta$  clearance.

Landreth believes that ApoE particles with abundant lipids bind to soluble A $\beta$  and shepherd the peptide to the surface of microglia, triggering internalization (*see Figure 1, "Boosting  $\beta$ -amyloid degradation"*).

Previously, researchers had believed that ApoE4 boosted the production and aggregation of A $\beta$ . "Until now, nobody had associated lipoprotein activity with A $\beta$  clearance," Landreth said.

Landreth also thinks the findings could help explain the mixed results of Wyeth and **Elan Corp. plc's** Phase II trial of bapineuzumab (AAB-001) antibody against A $\beta$ .<sup>6</sup> Although bapineuzumab failed to meet the primary endpoints of significant improvements base on the Alzheimer's Disease Assessment Scale (ADAS-cog) and on the Disability Assessment Scale for Dementia (DAD), the companies said the compound was effective among noncarriers of the *APOE4* gene.

Phase III trials of bapineuzumab are ongoing, including one in *APOE4* carriers and another in noncarriers.

Other academic researchers said Landreth's paper provides significant insight into the connection between lipid metabolism, proteolysis and AD.

"ApoE affecting AD has been around for many years, but nobody has made a link between ApoE and proteolysis" until now, said John Cirrito, research instructor in the Department of Neurology at **Washington University**. Cirrito, who has published several papers on ApoE in AD, told *SciBX* that the next question is how and where ApoE interacts with A $\beta$  or proteases. Knowing this could determine whether ApoE acts directly or recruits additional proteins that promote A $\beta$  degradation.

Malcolm Leissring, assistant professor at the **Mayo Clinic Jacksonville**, said the findings bring the neglected field of A $\beta$  proteolysis to the forefront of AD research. Until recently, he said, A $\beta$  degradation was considered a biochemical artifact rather than a natural clearance mechanism. Connecting the genetically well-studied ApoE lipoprotein

**"We found that you can increase the clearance of A $\beta$  by increasing the amount of ApoE."**

**—Gary Landreth,  
Case Western Reserve University**

to A $\beta$  proteolysis makes boosting degradation a more attractive therapeutic tactic.

## Good fat

Landreth's study also presents a new strategy for treating AD—boosting ApoE levels by activating the liver X receptor (LXR).

LXR has been studied in the liver, where it regulates the transcription of lipoprotein synthesis and cholesterol metabolism genes. However, Landreth's study suggests that LXR also can act in the brain.

The team treated AD mice with preclinical LXR agonists from **GlaxoSmithKline plc** and Wyeth, and observed higher ApoE levels, lower A $\beta$  levels and decreased plaque formation and inflammation in the brain than those seen in untreated controls. Moreover, AD mice treated with an LXR agonist showed better cognitive performance than untreated controls.

LXR agonists have been developed to treat hypercholesterolemia, but some have stalled in early-stage development due to safety concerns.

Anna Wilhelmsson, principal project manager at **Karo Bio AB**, told *SciBX* that Landreth's results help uncover the previously unknown mechanism underlying earlier studies of the effects of LXR activation on A $\beta$  levels.

“The demonstration that LXR activation stimulates A $\beta$  proteolysis rather than a decrease in A $\beta$  production is novel,” she said.

Last autumn, Karo Bio and partner Wyeth discontinued development of LXR-623, a modulator of LXR activity, after Phase I data in atherosclerosis suggested the compound had an unfavorable profile.

Landreth told *SciBX* that some of the current LXR agonists cause undesirable side effects such as elevated levels of triglycerides. “A lot

of development to iron out those effects” will be required before AD trials of LXR agonists can begin, he said.

Wilhelmsson agreed, noting that “the main challenge in developing LXR agonists for therapeutic use is still the increase in triglyceride synthesis that accompanies LXR stimulation.”

As an alternative to directly activating LXR, Landreth cited evidence that agonists of peroxisome proliferation-activated receptor- $\gamma$  (PPAR- $\gamma$ ) also lead to LXR activation, thus indirectly boosting ApoE levels.<sup>7</sup>

GlaxoSmithKline's rosiglitazone XR, an extended release formulation of the diabetes drug Avandia, is in Phase III trials to treat AD. An earlier Phase IIb trial in AD suggested that Avandia XR could benefit noncarriers of ApoE4.<sup>8</sup>

Landreth said he has not sought patents on his work, as the therapeutic use of LXR agonists is already extensively patented.

## Easy as PAI?

Meanwhile, a study in *PNAS* suggests that increasing the activity of the extracellular protease plasmin could be another way to clear out A $\beta$ . The work was conducted by a team led by Steven Jacobsen, associate director of neuroscience discovery at Wyeth.

Plasmin is known mostly for its thrombolytic activity in the peripheral bloodstream, where it cleaves a range of plasma proteins leading to the clearance of blood clots. Plasmin arises from proteolysis of its inactive precursor, plasminogen, by another protease called tissue plasminogen activator (tPA). Activase alteplase, a recombinant form of tPA, is marketed by **Genentech Inc.** to treat acute myocardial infarction, acute massive pulmonary embolism and acute ischemic stroke.

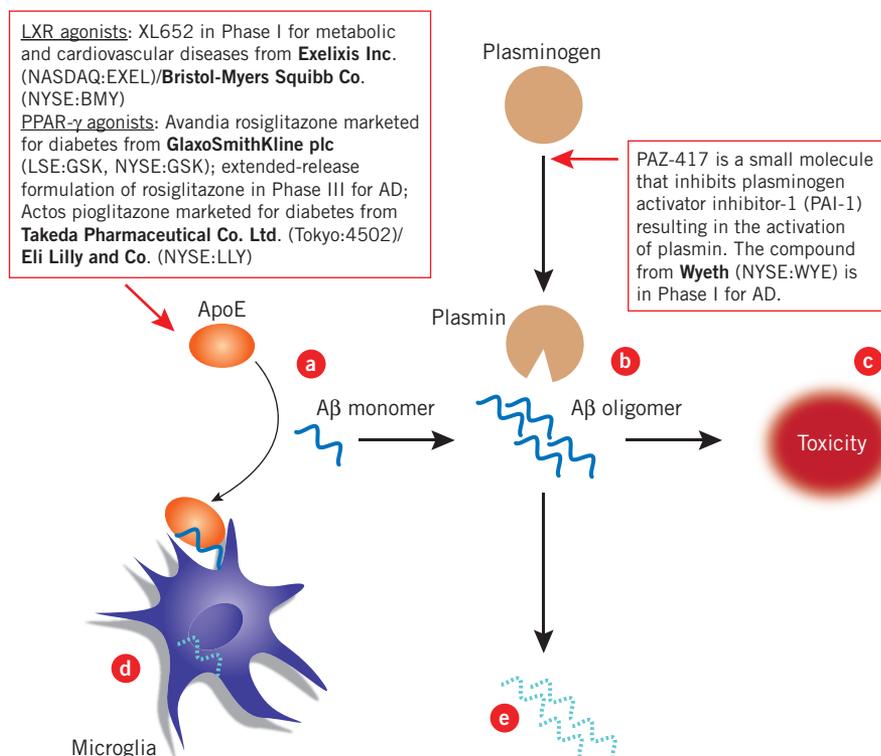
But plasmin also has an important function in brain physiology that may play a role in AD: it degrades A $\beta$  in the extracellular space

## Figure 1. Boosting $\beta$ -amyloid degradation.

In Alzheimer's disease (AD), extracellular  $\beta$ -amyloid (A $\beta$ ) monomers [a] accumulate into oligomers [b] that lead to plaque formation, inflammation and neurotoxicity [c]. Two new studies show how raising the activity of apolipoprotein E (ApoE) or stimulating proteases that degrade A $\beta$  could be useful therapeutic approaches.

Jiang *et al.* suggest that lipoprotein ApoE recruits soluble A $\beta$  to microglial cells, where it is degraded by intracellular and secreted proteases [d]. The researchers found that ApoE levels can be increased by stimulating the liver X receptor (LXR), suggesting the potential use of LXR agonists to treat AD. Additionally, peroxisome proliferation-activated receptor- $\gamma$  (PPAR- $\gamma$ ) activation has previously been found to turn on LXR, making compounds targeting PPAR- $\gamma$  potentially useful for treating the disease.

Jacobsen *et al.* show that increasing the activity of the protease plasmin can clear the brain of A $\beta$  oligomers [e].



surrounding the neurons. Previous work has shown that plasmin is less active in AD-stricken brains as a result of the overexpression of endogenous tPA antagonist PAI-1.<sup>9</sup>

Based on this information, Jacobsen's team reasoned that inactivating PAI-1 could raise plasmin activity, thus lowering A $\beta$  levels and preventing neurodegeneration. The premise behind the *in vitro* and mouse studies, he said, is that in AD, "unusually high levels of PAI-1 block the activation of plasmin, so that plasmin cannot cleave A $\beta$ ."

To test this hypothesis, Jacobsen's team mined Wyeth's compound library for PAI-1 inhibitors. The pharma company has published preclinical studies of earlier PAI-1 inhibitors in models for cardiovascular indications. The current development status of these compounds is undisclosed.<sup>10</sup>

The researchers found a small molecule PAI-1 inhibitor called PAZ-417 that crossed the blood-brain barrier and boosted tPA activity in the presence of PAI-1, thereby allowing tPA to activate plasmin.

In a cell-free plasmin activation assay, PAZ-417 stimulated the degradation of A $\beta$  compared with inhibitor-free control reactions.

PAZ-417 had a similar effect in two different mouse models of AD. A $\beta$ -overexpressing mice treated with PAZ-417 showed higher brain tPA activity, lower levels of A $\beta$  in the brain and serum, and better learning and memory than untreated controls.

"This is the first example of clearing up A $\beta$  by enhancing its metabolism," said Jacobsen.

Unlike NEP and IDE, plasmin can cut up oligomeric aggregates of A $\beta$ , according to Jacobsen. Thus, boosting plasmin activity could complement the degradation of soluble A $\beta$  described in Landreth's study (see **Figure 1**, "Boosting  $\beta$ -amyloid degradation").

Jacobsen told *SciBX* that inhibiting PAI-1 also complements Wyeth and Elan's immunotherapies, which are aimed at clearing oligomers of A $\beta$ . A Phase I trial of PAZ-417 to treat AD is ongoing. The company declined to comment on whether PAZ-417 and bapineuzumab could be used together.

## Getting specific

Steven Estus, associate professor of physiology at the **University of Kentucky**, said knockout studies indicate that plasmin is not strictly necessary for A $\beta$  clearance, but rather is one of several enzymes that can do the job. It is still unknown whether inhibiting PAI-1 stimulates other A $\beta$ -degrading proteases such as IDE.

Whereas A $\beta$ -generating intracellular proteases such as  $\beta$ -secretase and  $\gamma$ -secretase are targets for inhibition, extracellular proteases can be thought of as "potential therapeutic agents," according to Leissring. Knocking down intracellular proteolysis and boosting extracellular proteolysis are complementary strategies, he told *SciBX*.

Estus believes specificity is the primary concern in targeting proteases to promote A $\beta$  clearance. Increasing A $\beta$  proteolysis could be harmful if other brain proteins also get chewed up.

"NEP, IDE and plasmin are not specific for A $\beta$ ," said Estus. "Hence, this approach may hinge upon the therapeutic index of activators of these protease systems."

"There isn't an 'A $\beta$ -only' protease," agreed Cirrito. As boosting protease activity "could affect the whole body, you're opening yourself up to a lot of unpredictable side effects," he added.

Elevating tPA activity could pose safety concerns, according to academic researchers. Cambridge's Lomas noted that high tPA activity "could increase the risk of epilepsy." Likewise, Estus pointed to the "negative implications of tPA activation in stroke."

However, according to Jacobsen, his unpublished preclinical safety studies show PAZ-417 has no significant effects on clotting or cardiovascular activity. "We're not elevating tPA expression. We're taking very subtle levels of tPA and letting them do their job effectively," he said.

It is also unclear whether the beneficial effect of plasmin activation occurs exclusively in the brain.

"There is evidence that you can degrade A $\beta$  outside of the brain," said Louis Hersh, professor of molecular and cellular biochemistry at the University of Kentucky. He told *SciBX* that proteases or antibodies that deplete serum A $\beta$  could create a "one-way street" from the brain to the circulatory system to prevent a critical buildup of A $\beta$  in the brain.

Jacobsen believes that PAZ-417 acts primarily in brain. He noted that a chemically similar PAI-1 inhibitor that cannot cross the blood-brain barrier was not effective in AD mice, suggesting that only local inhibition of PAI-1 could be sufficient to stimulate A $\beta$  clearance.

Hersh has filed patents on the therapeutic use of IDE to treat AD and is negotiating with undisclosed companies to license his discoveries.

The technology in the Jacobsen study is patented by Wyeth and is not available for licensing, according to company spokesman Michael Lampe.

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**Elan Corp. plc** (NYSE:ELN), Dublin, Ireland  
**Cambridge Institute for Medical Research**, Cambridge, U.K.  
**Case Western Reserve University**, Cleveland, Ohio  
**Genentech Inc.** (NYSE:DNA), South San Francisco, Calif.  
**GlaxoSmithKline plc** (LSE:GSK; NYSE:GSK), London, U.K.  
**Karo Bio AB** (SSE:KARO), Stockholm, Sweden  
**Mayo Clinic Jacksonville**, Jacksonville, Fla.  
**University of Kentucky**, Lexington, Ky.  
**Washington University**, Saint Louis, Mo.  
**Wyeth** (NYSE:WYE), Madison, N.J.

# The path of Iressa resistance

By Michael J. Haas, Senior Writer

A report in the *Journal of Clinical Investigation* provides evidence that cancer cells develop resistance to Iressa gefitinib, an epidermal growth factor receptor inhibitor, by upregulating insulin-like growth factor-1.<sup>1</sup> Although the paper showed that the resistance can be overcome by inhibiting both receptors, companies working in the space had mixed views on whether the resistance mechanism is common to other epidermal growth factor receptor inhibitors and whether the findings would be widely applicable to human cancers.

There was agreement that the data bolster the rationale for using multiple growth factor inhibitors to treat cancer, and that it could provide markers to predict patient response.

The *JCI* team included researchers from **Harvard Medical School's Massachusetts General Hospital Cancer Center**, **Vanderbilt University School of Medicine** and the **University of Minnesota Cancer Center**. They were led by Jeffrey Engelman, instructor of medicine at Massachusetts General, and Carlos Arteaga, professor of medicine and cancer biology at Vanderbilt.

## Vive la résistance

Gefitinib is marketed as Iressa by **AstraZeneca plc** to treat metastatic or locally advanced non-small cell lung cancer (NSCLC).

Epidermal growth factor receptor (EGFR) inhibitors block a signaling cascade responsible for activating the phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt) pathway for cellular growth and survival. Iressa and the other approved small molecule EGFR inhibitor—Tarceva erlotinib from **OSI Pharmaceuticals Inc.**, **Genentech Inc.** and **Roche**—are effective in about 10–20% of lung cancer cases, but their effectiveness is limited or lost as resistance develops. A number of studies have linked about half of these resistant cancers to a key point mutation in the *EGFR* gene itself, but those reports did not account for why or how resistance occurs in the other half expressing the wild-type gene.<sup>2,3</sup>

At least three recent studies—two of them involving Engelman—found that Iressa-resistant cancers can upregulate c-Met receptor tyrosine kinase, thereby providing an alternative to EGFR signaling for PI3K and Akt activation. This alternative pathway was found in cancer cells with the mutant *EGFR* as well as in those with the wild-type gene.<sup>4-6</sup>

The current *JCI* study looked for potential mechanisms of resistance in Iressa-resistant cancer cells having only the wild-type gene.

First, the Engelman-Arteaga team developed Iressa-resistant cells by growing squamous epithelial cells in increasing concentrations of Iressa over a period of several months.

They found that insulin receptor substrate 1 (IRS1)—a substrate of the insulin-like growth factor-1 (IGF-1) receptor that activates the

PI3K and Akt pathway—was upregulated in these cells. This led the team to hypothesize that the resistant cells had lost sensitivity to Iressa because of activation of the IGF-1 receptor and IRS-1 signaling cascade, which Iressa does not target.

The team confirmed this hypothesis by showing that treatment of the resistant cells with a combination of Iressa and an IGF-1 receptor inhibitor restored sensitivity to Iressa.

Subsequent gene profiling and small hairpin RNA experiments indicated that upregulation of the IGF-1 receptor resulted primarily from decreased expression of its negative regulator, insulin-like growth factor binding protein-3 (IGFBP3). Indeed, Iressa plus recombinant IGFBP3 restored Iressa sensitivity.

In addition, head and neck cancer cells sensitive to Iressa did not develop resistance when treated with a combination of Iressa and a small molecule IGF-1 receptor inhibitor.

The team obtained comparable results *in vitro* with Erbitux cetuximab, an anti-EGFR antibody marketed by **ImClone Systems Inc.**, **Bristol-Myers Squibb Co.** and **Merck KGaA**. Cetuximab-resistant squamous epithelial cells developed by the team also downregulated IGFBP3 and were sensitive to the combina-

tion of cetuximab and a small molecule IGF-1 receptor inhibitor.

Lastly, seven mice bearing xenograft Iressa-resistant tumors were treated with a combination of Iressa and a small molecule IGF-1 receptor inhibitor. All of the mice responded to therapy, and only one had a tumor recur—two months after treatment was stopped. By contrast, mice treated with Iressa or the IGF-1 receptor inhibitor alone experienced either tumor progression or tumor recurrence.

Collectively, the results indicate that cancer cells can develop resistance to Iressa by activating an alternative growth factor signaling cascade—the IGF-1 receptor and IRS-1 pathway—and that combining Iressa with an IGF-1 receptor inhibitor can overcome this resistance.

Neither Engelman nor Arteaga was available for comment. But Geoffrey Allan, president and CEO of **Insmmed Inc.**, told *SciBX*: “This paper is the first to provide an in-depth look at the mechanism of resistance to epidermal growth factor receptor inhibitors. It provides concrete, detailed evidence for something that was postulated on the basis of earlier—but limited—studies.”

Insmmed's rhIGFBP-3 (recombinant IGFBP3), is in preclinical development for breast, liver, colon, prostate and ovarian cancers. The company recently completed a Phase I trial of INMS-18, a small molecule inhibitor of IGF-1 receptor and hairy-related 2 (HER2), and plans to take that compound into a Phase II trial to treat prostate cancer in the near future.

David Kirn, president and CEO of **Jennerex Biotherapeutics Inc.**, agreed with Allan. “Previous studies have shown resistance to result from specific mutations in the EGF receptor gene itself,” he said. “This is the first clear evidence of a downstream compensatory mechanism of resistance.”

Jennerex's JX-594, a recombinant vaccinia virus that targets EGFR, is in Phase II testing to treat liver cancer and melanoma, and Phase I/II testing to treat lung cancer.

“This paper is the first to provide an in-depth look at the mechanism of resistance to epidermal growth factor receptor inhibitors.”

—Geoffrey Allan, Insmmed Inc.

## Rational combination

Company and institution representatives contacted by *SciBX* agreed that the *JCI* paper supports the use of a combination of growth factor inhibitors to treat cancer. Opinions differed, however, on which cancers and compounds might trigger the resistance described in the paper.

“There is widespread understanding that monotherapy is not the optimum pathway because of the complexity of cancer,” said Elwyn Loh, executive director for global development and global development leader for growth regulation products at **Amgen Inc.** “In that context, this paper provides a real mechanistic framework to think about this combination of agents.”

But Loh said it was not yet clear whether the *JCI* results were relevant in any other cancers. “The paper offers suggestive evidence that one can apply the findings to a spectrum of human cancers,” he said. “But we need to do experiments in people to confirm this.”

He also wanted to know whether the mechanism of resistance described in the *JCI* article is the primary pathway of Iressa resistance. “Is this mechanism dominant across many different cell lines—or just one of multiple pathways of resistance?” he asked.

Loh said the answer would indicate whether a combination of EGFR and IGF-1 receptor inhibitors would be widely useful in overcoming Iressa resistance.

Amgen’s Vectibix panitumumab anti-EGFR antibody is marketed for metastatic colorectal cancer. The company’s AMG479 anti-IGF-1 receptor antibody is in Phase II trials to treat breast cancer, pancreatic cancer and Ewing’s sarcoma. Amgen plans to start additional Phase II studies of AMG479 in other cancers, including NSCLC, but has not announced timelines.

“As far as we’re concerned, this mechanism of resistance is one of many,” said Iain Hutcheson, senior research associate at **Cardiff University**. He added that cancers can resist Iressa by using multiple signaling routes to maintain PI3K and Akt activation, and that targeting PI3K and Akt, instead of molecules further upstream, might be a better strategy.

By contrast, Insmed’s Allan thought the mechanism would be generally applicable to tumors resistant to EGFR inhibitors. “Insmed did an *in vitro* study in breast cancer cells of its recombinant IGF1R in combination with Herceptin, and the combination seemed to give better tumor control,” he said.<sup>7</sup>

That Insmed research collaboration—which included scientists from **McGill University**, the **University of California, Los Angeles**’s David Geffen School of Medicine and **Xanthus Pharmaceuticals Inc.** (formerly Xanthus Life Sciences)—found that the IGF-1 receptor was highly expressed in breast cancer cells that had developed resistance to Herceptin. But the combination of Herceptin and recombinant IGF1R restored the cancer cells’ sensitivity to Herceptin, both *in vitro* and in xenograft mouse tumors.

Genentech markets its Herceptin trastuzumab anti-HER2 antibody in the U.S. to treat breast cancer. Roche markets the drug elsewhere.

Allan noted that the *JCI* report is also consistent with epidemiological data showing that levels of IGF1R are inversely related to the incidence of breast cancer.<sup>8,9</sup>

## Clinical strategy

David Epstein, SVP of oncology research at OSI, said therapeutic

application of the *JCI* findings would take time to assess.

“The finding that concurrent inhibition of IGF-1 receptor abrogates the emergence of resistance to EGFR tyrosine kinase inhibitors is a new observation,” Epstein said. But he added that it remains to be seen whether cancers would develop this mechanism of resistance in response to other small molecules and antibodies against EGFR and whether it would occur in a broad range of tumors.

In addition to Tarceva, OSI’s OSI-906, an IGF-1 receptor inhibitor, is in Phase I trials to treat advanced solid tumors. Epstein said OSI is planning Phase II trials that will combine the compound with Tarceva and other signal transduction inhibitors.

Although the prevalence of this resistance mechanism will have to be established, Epstein suggested the *JCI* findings might yield useful biomarkers.

Jennerex’s Kirn agreed. “With a screen based on IGF1R levels, you could predict which patients are likely to develop, or are developing, resistance” to treatment with Iressa, he said. Studies to examine this potential correlation could begin right away, he added.

Kirn noted more data on the safety of the inhibitor combination would be needed. A key question to answer, he said, is whether normal cells are “as dependent, or less dependent, than cancer on the EGFR receptor and IGF receptor pathways.” If normal cells depend on both, the combination of inhibitors might have increased toxicity, he said.

Uma Sundaram, senior licensing associate at Massachusetts General, said the findings reported in *JCI* are patented and available for licensing.

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**Amgen Inc.** (NASDAQ:AMGN), Thousand Oaks, Calif.  
**AstraZeneca plc** (LSE:AZN; NYSE:AZN), London, U.K.  
**Bristol-Myers Squibb Co.** (NYSE:BMJ), New York, N.Y.  
**Cardiff University**, Cardiff, U.K.  
**Genentech Inc.** (NYSE:DNA), South San Francisco, Calif.  
**Harvard Medical School**, Boston, Mass.  
**ImClone Systems Inc.** (NASDAQ:IMCL), New York, N.Y.  
**Insmed Inc.** (NASDAQ:INSM), Richmond, Va.  
**Jennerex Biotherapeutics Inc.**, San Francisco, Calif.  
**Massachusetts General Hospital Cancer Center**, Charlestown, Mass.  
**McGill University**, Montreal, Quebec, Canada  
**Merck KGaA** (Xetra:MRK), Darmstadt, Germany  
**OSI Pharmaceuticals Inc.** (NASDAQ:OSIP), Melville, N.Y.  
**Roche** (SWX:ROG), Basel, Switzerland  
**University of California, Los Angeles**, Calif.  
**University of Minnesota Cancer Center**, Minneapolis, Minn.  
**Vanderbilt University School of Medicine**, Nashville, Tenn.  
**Xanthus Pharmaceuticals Inc.**, Cambridge, Mass.

## 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   |
|---------------|---|---|--|---|
| <b>Cancer</b> |   |   |  |   |
| Brain cancer  | Chromodomain helicase DNA binding protein 5 (CHD5)  | Studies in cell culture and in mice suggest that activation or overexpression of tumor suppressor CHD5 could help treat neuroblastoma. In neuroblastoma cell lines, CHD5 overexpression significantly minimized the size and number of colonies compared with what was seen in control lines receiving antisense CHD5 ( $p < 0.001$ ). In mice, CHD5-transfected tumors had significantly lower growth and volume than antisense CHD5-transfected tumors in controls ( $p \leq 0.01$ ). In neuroblastoma patients, CHD5 expression was significantly associated with event-free and overall survival ( $p < 0.001$ ). Next steps include studies to validate the prognostic relevance of CHD5 in neuroblastoma patients. At least seven companies have compounds in clinical and preclinical testing to treat neuroblastoma.  | Provisional patent application filed for CHD5 and its associated use; available for licensing through The Joseph Stokes Jr. Research Institute Office of Technology Transfer | Fujita, T. <i>et al. J. Natl. Cancer. Inst.</i> ; published online June 24, 2008; doi:10.1093/jnci/djn176<br><b>Contact:</b> Garrett M. Brodeur, The Children's Hospital of Philadelphia, Philadelphia, Pa.<br>e-mail: <a href="mailto:brodeur@chop.edu">brodeur@chop.edu</a>   |
| Cancer        | Epidermal growth factor receptor (EGFR); insulin-like growth factor-1 receptor (IGF1R); insulin-like growth factor binding protein-3 (IGFBP3) | Studies in cell culture and in mice suggest that combining gefitinib with an IGF1R inhibitor could be useful for treating gefitinib-resistant tumors. Researchers found that IGFBP3 was downregulated in gefitinib-resistant cancer cells. This downregulation allowed increased expression of IGF1R, opening an alternative pathway to phosphoinositide 3-kinase and protein kinase B activation for continued cell survival and proliferation. The addition of recombinant IGFBP3 restored the sensitivity of the cancer cells to gefitinib. In mice bearing gefitinib-resistant xenografts, treatment with either a small molecule or an mAb targeting IGF1R overcame resistance and prevented tumor recurrence. Further work will investigate this mechanism in cancer patients treated with gefitinib and/or other EGFR tyrosine kinase inhibitors and will also study how prevalent the mechanism is in human cancers.<br>Partners AstraZeneca plc and Teva Pharmaceutical Industries Ltd. market Iressa gefitinib to treat non-small cell lung cancer (NSCLC). Many other companies market or are developing EGFR inhibitors to treat a wide range of cancers. Insmed Inc. has a recombinant IGFBP3 in clinical development to treat prostate and other cancers. More than a dozen companies are developing small molecules and/or mAbs targeting IGF1R to treat cancer ( <i>see The path of Iressa resistance, page 10</i> ). | Patented; available for licensing  | Guix, M. <i>et al. J. Clin. Invest.</i> ; published online June 20, 2008; doi:10.1172/JCI34588<br><b>Contacts:</b> Carlos L. Arteaga, Vanderbilt University Medical Center, Nashville, Tenn.<br>e-mail: <a href="mailto:carlos.arteaga@vanderbilt.edu">carlos.arteaga@vanderbilt.edu</a><br><b>Contact:</b> Jeffrey A. Engelman, Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown, Mass.<br>e-mail: <a href="mailto:jengelman@partners.org">jengelman@partners.org</a> |

## This week in therapeutics (continued)

| Indication   | Target/marker/pathway  | Summary  | Licensing status   | Publication and contact information   |
|--------------|--|--|--|---|
| Cancer       | Phosphoinositide 3-kinase, catalytic, $\beta$ -peptide (PIK3CB; PI3K-p110 $\beta$ ; p110 $\beta$ ) | Studies in cell lines and mice suggest that antagonizing p110 $\beta$ could be useful for treating cancer. In immortalized mouse embryonic fibroblasts (MEFs), p110 $\beta$ knockout lowered oncogene-driven transformation compared with that seen in wild-type MEFs. In a mouse model of prostate cancer, prostate-specific p110 $\beta$ knockout resulted in significantly less prostatic epithelial neoplasia than that seen in wild-type controls ( $p < 0.001$ ). Further studies are necessary to evaluate p110 $\beta$ tumor models and specific p110 $\beta$ inhibitors. No fewer than five companies have compounds inhibiting PI3K to treat cancer in clinical and preclinical testing.   | Research not patented; available for licensing   | Jia, S. <i>et al. Nature</i> ; published online June 26, 2008; doi:10.1038/nature07091<br><b>Contact:</b> Jean J. Zhao, Harvard Medical School, Boston, Mass.<br>e-mail: <a href="mailto:jean_zhao@dfci.harvard.edu">jean_zhao@dfci.harvard.edu</a><br><b>Contact:</b> Thomas M. Roberts, same affiliation as above<br>e-mail: <a href="mailto:thomas_roberts@dfci.harvard.edu">thomas_roberts@dfci.harvard.edu</a> |
| Cancer       | Unknown  | Studies in mice suggest that TNP-470, an oral fumagillin analog, could help treat cancer and prevent metastasis. The compound was produced by conjugating TNP-470 to monomethoxy-polyethyleneglycol-poly(lactic acid) (mPEG-PLA) to form nanoscale polymeric micelles. In mice, oral TNP-470 significantly inhibited tumor growth compared with that seen in mice that received control vehicle mPEG-PLA ( $p < 0.05$ ). In mice injected with tumor cells, oral TNP-470 prevented liver metastasis and prolonged survival compared with what was seen in untreated mice bearing tumors. Next steps include studying oral TNP-470 in mouse xenograft models of breast and brain cancer. Caplostatin, the conjugation of TNP-470 to <i>N</i> -(2-hydroxypropyl)methacrylamide (HPLA), is in preclinical development by SynDevRx Inc. to treat cancer.   | Patent application filed; SynDevRx has an exclusive option to license the oral formulation of TNP-470 (under the name Lodamin)   | Benny, O. <i>et al. Nat. Biotechnol.</i> ; published online June 29, 2008; doi:10.1038/nbt1415<br><b>Contact:</b> Ofra Benny, Harvard Medical School, Boston, Mass.<br>e-mail: <a href="mailto:ofra.bennyratsaby@childrens.harvard.edu">ofra.bennyratsaby@childrens.harvard.edu</a>   |
| Colon cancer | Guanylyl cyclase 2C (heat-stable enterotoxin receptor; GUCY2C)                                     | Studies in mice suggest that immunization with GUCY2C could help treat or prevent metastatic colon cancer. In healthy mice, immunization with GUCY2C-expressing viral vectors before challenge with GUCY2C-expressing mouse colon cancer cells minimized metastasis to the liver and lungs compared with that seen in mock-treated control mice ( $p = 0.008$ and $p < 0.001$ , respectively). In mice with established metastases, median survival was 38 days for immunized mice compared with 29 days for untreated mice ( $p = 0.024$ ). The antitumor and pro-survival effects occurred without autoimmune reactions. Completion of safety and efficacy studies in animals, as well as GMP studies, are necessary before the vaccine enters the clinic. Castillo Pharmaceuticals Inc.'s SP-304, a GUCY2C agonist, is in Phase I testing to treat irritable bowel syndrome (IBS). Ironwood Pharmaceuticals and Forest Laboratories are developing MD-1100, a GUCY2C agonist that is in Phase II testing to treat constipation and IBS. | Patent applications submitted; Targeted Diagnostics & Therapeutics Inc. has exclusive worldwide rights to the GUCY2C cancer technology and has sublicensed most of the cancer applications to Millennium Pharmaceuticals Inc.; some of the vaccine applications available for sublicensing | Snook, A. <i>et al. J. Natl. Cancer Inst.</i> ; published online June 24, 2008; doi:10.1093/jnci/djn178<br><b>Contact:</b> Scott A. Waldman, Thomas Jefferson University, Philadelphia, Pa.<br>e-mail: <a href="mailto:scott.waldman@jefferson.edu">scott.waldman@jefferson.edu</a>   |

## This week in therapeutics (continued)

| Indication                  | Target/marker/pathway  | Summary   | Licensing status  | Publication and contact information   |
|-----------------------------|--|---|---|---|
| Ovarian cancer              | <i>Excision repair cross-complementing rodent repair deficiency, complementation group 5 (ERCC5)</i>                               | A genome-wide association study suggests that <i>ERCC5</i> could be a useful biomarker of ovarian cancer response to platinum-based chemotherapy. <i>ERCC5</i> is part of the nucleotide excision repair pathway that removes platinum-DNA adducts. In epithelial ovarian cancer patients treated with surgery followed by platinum-based chemotherapy, loss of heterozygosity of <i>ERCC5</i> was associated with improved progression-free survival (PFS) compared with what was seen in patients without loss of heterozygosity. In the same patients, downregulation of <i>ERCC5</i> expression was associated with better PFS and overall survival compared with that of patients who had upregulated <i>ERCC5</i> . Next steps include additional testing to validate the findings.   | Not patented; unlicensed  | Walsh, C. <i>et al. J. Clin. Oncol.</i> ; published online June 18, 2008;<br>doi: 10.1200/JCO.2007.13.5806<br><b>Contact:</b> Christine Walsh, Cedars-Sinai Medical Center, Los Angeles, Calif.<br>e-mail:<br><a href="mailto:walshc@cshs.org">walshc@cshs.org</a>        |
| <b>Endocrine disease</b>    |  |   |   |   |
| Obesity; metabolic syndrome | <i>Lipoprotein lipase (Lpl); <math>\beta</math>-lactamase (Lactb); protein phosphatase 1 (formerly 2C)-like (Ppm1L; Ppm1-like)</i> | An integrative genomic study of mice suggests that proteins involved with lipid metabolism in macrophages could be targets to treat obesity and metabolic syndrome. Combined analysis of gene expression variation and quantitative trait loci identified <i>Lpl</i> , <i>Lactb</i> and <i>Ppm1l</i> as key members of a macrophage-associated gene cluster that underlies variation in obesity-related traits. <i>Lpl</i> -heterozygous and <i>Lactb</i> -overexpressing mice showed 22% and 20% higher fat-mass-to-lean-mass ratios than wild-type littermates. <i>Ppm1</i> knockouts showed 19.3% higher weight and 46.7% higher fat mass than homozygous littermates. Merck & Co. Inc. has compounds that target proteins identified in this study in discovery stages to treat obesity and metabolic syndrome (see <b>Merck's Rosetta stone, page 1</b> ). | Patents filed on treatments targeting these genes; licensing status undisclosed | Chen, Y. <i>et al. Nature</i> ; published online March 16, 2008;<br>doi:10.1038/nature06757<br><b>Contact:</b> Eric Schadt, Rosetta Inpharmatics LLC, Seattle, Wash.<br>e-mail:<br><a href="mailto:eric_schadt@merck.com">eric_schadt@merck.com</a>                       |
| <b>Neurology</b>            |  |   |   |   |
| Alzheimer's disease (AD)    | $\beta$ -amyloid (A $\beta$ )  | Studies <i>in vitro</i> and in mice suggest that the tetravalent guanlylhydrazone compound CNI-1493 could be useful for treating AD. In a mouse model of AD, CNI-1493 significantly reduced A $\beta$ plaque area in the cortex by 70% and in the hippocampus by 86% compared with what was seen in vehicle-treated controls ( $p=0.016$ for both). CNI-1493 also improved the object recognition memory performances of the mice compared with those of vehicle-treated controls. Next steps include determining the mechanism of neuroprotection and studies in additional mouse models of AD. Cytokine PharmaSciences Inc.'s Semapimod, formerly CN-1493, is in a Phase II trial to treat pancreatitis, a Phase II trial to treat Crohn's disease and a Phase I trial as an adjunct to IL-2 in cancer.   | Patent application filed; licensed to Cytokine PharmaSciences                   | Bacher, M. <i>et al. J. Exp. Med.</i> ; published online June 23, 2008;<br>doi:10.1084/jem.20060467<br><b>Contact:</b> Yousef Al-Abed, The Feinstein Institute for Medical Research, Manhasset, N.Y.<br>e-mail:<br><a href="mailto:yalabel@nshs.edu">yalabel@nshs.edu</a> |

## This week in therapeutics (continued)

| Indication  | Target/marker/<br>pathway  | Summary   | Licensing<br>status                              | Publication and contact<br>information  |
|---|--|---|--|---|
| <b>Transplantation</b>                              |  |   |  |   |
| Graft-versus-host disease (GvHD)                    | Indoleamine-pyrrole 2,3-dioxygenase (INDO); histone deacetylase (HDAC)                                     | A study in mice suggests that HDAC inhibitors may be useful for treating GvHD. In a mouse model of GvHD, injection of dendritic cells (DCs) treated <i>ex vivo</i> with the HDAC inhibitor SAHA significantly improved survival and lowered clinical disease at day 21 compared with injection of untreated DCs ( $p < 0.01$ and $p < 0.05$ , respectively). Cell-culture studies showed that the immunosuppressive effect of HDAC inhibitors was mediated by INDO. Next steps include studying the mechanism by which HDAC affects INDO expression and investigating the immunomodulatory effects of HDAC inhibitors in clinical trials.<br>Merck & Co. Inc. markets Zolinza SAHA for cutaneous T cell lymphoma (CTCL).  | Not patented; unavailable for licensing          | Reddy, P. <i>et al.</i> <i>J. Clin. Invest.</i> ; published online June 20, 2008; doi:10.1172/JCI34712<br><b>Contact:</b> Pavan Reddy, University of Michigan Cancer Center, Ann Arbor, Mich. e-mail: <a href="mailto:reddypr@umich.edu">reddypr@umich.edu</a>          |
| <b>Various</b>                                      |  |   |  |   |
| Coronary artery disease (CAD); hypercholesterolemia | <i>Sortilin 1</i> ( <i>SORT1</i> ); <i>Cadherin EGF LAG seven-pass G-type receptor 2</i> ( <i>CELSR2</i> ) | An integrative genomic study of human and mouse liver samples identified new candidate genes that influence CAD and hypercholesterolemia. The combination of gene expression profiling and genome-wide association analysis identified <i>SORT1</i> and <i>CELSR2</i> as quantitative trait loci underlying variation in low-density lipoprotein (LDL) levels and susceptibility to CAD. In mice, <i>SORT1</i> transcript levels were negatively correlated with plasma LDL ( $r = -0.50$ , $p < 10^{-16}$ ), whereas <i>CELSR2</i> transcription was positively correlated ( $r = 0.40$ , $p = 3.23 \times 10^{-13}$ ). Next steps include validating <i>SORT1</i> and <i>CELSR2</i> as targets using animal models of CAD and hypercholesterolemia (see <b>Merck's Rosetta stone, page 1</b> ). | Patent and licensing status undisclosed          | Schadt, E. <i>et al.</i> <i>PLOS Biol.</i> ; published online May 6, 2008; doi:10.1371/journal.pbio.0060107<br><b>Contact:</b> Eric Schadt, Rosetta Inpharmatics LLC, Seattle, Wash. e-mail: <a href="mailto:eric_schadt@merck.com">eric_schadt@merck.com</a>           |
| Incontinence, diabetes; obesity                     | Adrenergic receptor $\beta 3$ (ADRB3)  | SAR studies identified a class of biphenyl benzoic acid derivatives that may be useful for treating metabolic diseases and incontinence. In a canine model of overactive bladder, one of the analogs led to a dose-dependent decrease in intravesical pressure compared with what was seen using previously developed compounds. Researchers did not disclose next steps.<br>MN-246, an ADRB3 agonist from Mitsubishi Tanabe Pharma Corp., is in Phase I testing for incontinence.  | Compounds patented; licensing status undisclosed | Imanishi, M. <i>et al.</i> <i>J. Med. Chem.</i> ; published online June 14, 2008; doi:10.1021/jm8000345<br><b>Contact:</b> Kouji Hattori, Astellas Pharma Inc., Ibaraki, Japan e-mail: <a href="mailto:kouji.hattori@jp.astellas.com">kouji.hattori@jp.astellas.com</a> |

## 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 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 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   |
|--|--|---|---|
| Aptamer-mediated biomarker discovery (AptaBiD)   | An <i>in vitro</i> study suggests that AptaBiD could be useful for identifying cell-surface biomarkers associated with multiple diseases. The technique uses aptamer libraries to differentially select cell-surface biomarkers, which are subsequently isolated from target cells and identified using mass spectrometry. As proof of principle, the technique identified biomarkers that differentiated mature dendritic cells from immature ones. Further validation of the method and comparison with conventional approaches are necessary.   | Provisional patent application filed in the U.S.; available for licensing worldwide                 | Berezovski, M. <i>et al. J. Am. Chem. Soc.</i> ; published online June 17, 2008; doi:10.1021/ja801951p<br><b>Contact:</b> Sergey N. Krylov, York University, Toronto, Ontario, Canada<br>e-mail: <a href="mailto:skrylov@yorku.ca">skrylov@yorku.ca</a>       |
| Chemically mediated somatic cell reprogramming   | Cell-culture studies suggest that inhibitors of chromatin-modifying enzymes can increase the efficiency with which somatic cells are reprogrammed to pluripotent stem cells, providing a potential boon to the development of stem cell-based therapies. In mouse embryonic fibroblasts expressing four transcription factors ( <i>Oct4</i> , <i>Sox2</i> , <i>Klf4</i> and <i>c-Myc</i> ) that are sufficient to induce pluripotency, small molecule inhibitors of DNA methyltransferase or histone deacetylase (HDAC) significantly increased reprogramming efficiency compared with that seen in dimethyl sulfoxide (DMSO)-treated controls ( $p < 0.05$ ). The HDAC inhibitor valproic acid (VPA) showed the strongest effect, with an increase of over 100-fold in programming efficiency compared with that seen in DMSO-treated controls ( $p < 0.001$ ). Next steps include assessing VPA's effects in human somatic cells and conducting a high throughput screen of small molecule libraries to identify other candidates for chemical-based cellular reprogramming. | Patent pending for VPA effects on reprogramming; available for licensing through Harvard University | Huangfu, D. <i>et al. Nat. Biotechnol.</i> ; published online June 22, 2008; doi:10.1038/nbt1418<br><b>Contact:</b> Douglas A. Melton, Harvard University, Cambridge, Mass.<br>e-mail: <a href="mailto:dmelton@harvard.edu">dmelton@harvard.edu</a>           |
| Human cell lines for <i>in vitro</i> study of primary systemic amyloidosis   | The ALMC-1 and ALMC-2 human cell lines may be useful tools for developing therapies to treat primary systemic amyloidosis. ALMC-1 was established from a bone marrow sample taken from a patient prior to relapse into symptomatic multiple myeloma (MM), whereas ALMC-2 was established from a sample taken from the same patient after symptomatic relapse. Both cell lines had morphologies similar to primary patient plasma cells and secreted $\gamma$ -light chain proteins ( $\gamma$ -LC) that formed amyloid fibrils. ALMC-2 cells had higher rates of IgG and free $\gamma$ -LC secretion than ALMC-1 cells. Next steps include using the cell lines as screening tools for compounds that inhibit amyloid formation and developing an animal model of amyloidosis. At least three companies have compounds in Phase II and Phase III testing for amyloidosis.  | Not patented; available for licensing through Mayo Clinic Office of Intellectual Property           | Arendt, B.K. <i>et al. Blood</i> ; published online June 20, 2008; doi:10.1182/blood-2008-03-143040<br><b>Contact:</b> Diane F. Jelinek, Mayo Clinic, Rochester, Minn.<br>e-mail: <a href="mailto:jelinek.diane@mayo.edu">jelinek.diane@mayo.edu</a>          |
| Integrative genomic study to identify gene expression networks that show a causal relationship with obesity-related traits | An integrative genomic study of human tissue samples identified a human macrophage-enriched metabolic network (MEMN) that could be used to identify genetic determinants of obesity and therapeutic response. Combined analysis of gene expression, quantitative trait loci and SNP genome-wide linkage identified a subnetwork of macrophage-associated genes in the human MEMN that were associated with variation in body mass index, percentage body fat and waist-to-hip ratio. The human MEMN subnetwork showed significant overlap with the previously described mouse MEMN subnetwork that was enriched with genes involved in inflammatory response and macrophage-activation pathways. Next steps include selecting individual target genes for validation in experimental models of obesity. deCODE genetics Inc. and Merck & Co. Inc. ended a partnership to discover obesity targets in 2005 ( <i>see Merck's Rosetta stone, page 1</i> ).  | General use patents filed jointly by deCODE and Merck; licensing status undisclosed                 | Emilsson, V. <i>et al. Nature</i> ; published online March 16, 2008; doi:10.1038/nature06758<br><b>Contact:</b> Kari Stefansson, deCODE genetics Inc., Reykjavik, Iceland<br>e-mail: <a href="mailto:kari.stefansson@decode.is">kari.stefansson@decode.is</a> |

## This week in techniques (continued)

| Approach  | Summary  | Licensing status  | Publication and contact information  |
|---|--|---|--|
| Integrative genomics analysis in yeast  | An integrative genomic study in yeast points to master regulatory genes that might be targeted to treat multiple metabolic and developmental disorders. Genotype and gene expression analysis of 112 offspring from a cross of two unrelated strains identified a panel of hotspot loci with broad effects on gene expression. Combined with pre-existing data about transcription factor binding sites and protein-protein interactions, these data predicted candidate genes that regulate the expression of other genes. Several candidates were experimentally confirmed as previously unknown metabolic and stress-response regulators. Concurrent studies in mice and humans extend this method to identifying genes that regulate human disease (see <b>Merck's Rosetta stone</b> , page 1).                              | Not patented; licensing status undisclosed  | Zhu, J. <i>et al. Nat. Genet.</i> ; published online June 15, 2008; doi:10.1038/ng.167<br><b>Contact:</b> Eric Schadt, Rosetta Inpharmatics LLC, Seattle, Wash.<br>e-mail: <a href="mailto:eric_schadt@merck.com">eric_schadt@merck.com</a>  |
| NMR-based screen for identifying naturally occurring cancer therapeutics                | A multistep NMR screen for ligands of the nucleic acid G-quadruplex motif could be useful for identifying cancer compounds in plant extracts. G-quadruplex motifs have been implicated in regulatory processes, including carcinogenesis. The method uses proton NMR spectroscopy to identify the presence or absence of G-quadruplex ligands in the extract. Next, 2D NMR is used to determine the structure of the ligands. A proof-of-concept study using ethanol and water extracts of <i>Phellodendron chinense</i> Schneid cortexes and <i>Coptis chinensis</i> Franch rhizomes showed that a 0.06% mass content of G-quadruplex ligand was detectable with the technique. Next steps include using the NMR method to determine the structure of G-quadruplex ligands from traditional medicines and other plant extracts. | Not patented; licensing status not applicable   | Zhou, Q. <i>et al. Angew. Chem. Int. Ed.</i> ; published online June 18, 2008; doi:10.1002/anie.200800913<br><b>Contact:</b> Yalin Tang, Chinese Academy of Sciences, Beijing, China<br>e-mail: <a href="mailto:tangyl@iccas.ac.cn">tangyl@iccas.ac.cn</a>   |
| Synthetic attenuated virus engineering (SAVE)   | Studies in cell culture and mice suggest that SAVE could be a useful strategy for <i>de novo</i> production of live attenuated viral vaccines. Multiple suboptimal synonymous codons were engineered into regions of the poliovirus genome that code for the capsid structural protein. In cell culture, the resulting poliovirus strain had substantially lower virulence than wild-type poliovirus. In mice, the new strain elicited an immune response that protected the mice against death or paralysis following challenge with wild-type poliovirus. Next steps include validation studies using SAVE in other viruses.   | Patent pending for SAVE; licensing status undisclosed                                   | Coleman, J.R. <i>et al. Science</i> ; published online June 26, 2008; doi:10.1126/science.1155761<br><b>Contact:</b> Steffen Mueller, State University of New York at Stony Brook, Stony Brook, N.Y.<br>e-mail: <a href="mailto:smueller@ms.cc.sunysb.edu">smueller@ms.cc.sunysb.edu</a><br><b>Contact:</b> Bruce Fletcher, same affiliation as above<br>e-mail: <a href="mailto:bfletcher@ms.cc.sunysb.edu">bfletcher@ms.cc.sunysb.edu</a><br><b>Contact:</b> Steve Skiena, same affiliation as above<br>e-mail: <a href="mailto:skiena@cs.sunysb.edu">skiena@cs.sunysb.edu</a> |
| Transplantation of allogeneic cytokine-induced killer (CIK) cells for antitumor therapy | A study in mice suggests that transplanted allogeneic CIK cells could be useful for treating cancer while causing minimal graft-versus-host disease (GvHD). In tumor-bearing mice, treatment with CIK cells and T cell-depleted bone marrow (TCD-BM) minimized tumor growth compared with that seen in tumor-bearing controls that received only TCD-BM. In irradiated mice receiving rescue bone marrow transplants, co-treatment with CIK cells resulted in 100% survival compared with no survival for mice receiving rescue bone marrow transplants plus splenocytes. Mice treated with CIK cells had minimal GvHD, whereas most mice that received splenocytes developed fatally acute GvHD. The approach is in clinical testing for use in autologous and allogeneic transplantation therapies.                            | Aspects of work have been patented; available for licensing through Stanford University | Nishimura, R. <i>et al. Blood</i> ; published online June 18, 2008; doi:10.1182/blood-2007-06-092817<br><b>Contact:</b> Robert S. Negrin, Stanford University, Stanford, Calif.<br>e-mail: <a href="mailto:negrs@stanford.edu">negrs@stanford.edu</a>  |



|                                    |         |                              |       |  |                              |       |
|------------------------------------|---------|------------------------------|-------|--|------------------------------|-------|
| <b>D</b>                           |         | <b>I</b>                     |       | Meta-tetra(hydroxyphenyl)              | Ppm1L                        | 3,14  |
| Dimethyl sulfoxide                 | 16      | IDE                          | 7     | chlorine                               | Protein kinase B             | 10,12 |
| DMSO                               | 16      | IGF-1                        | 10    | MN-246                                 | Protein phosphatase 1        |       |
| DNA methyltransferase              | 16      | IGF1R                        | 12    | Monomethoxy-                           | (formerly 2C)-like           | 3,14  |
| <b>E</b>                           |         | IGFBP3                       | 10,12 | polyethyleneglycol-polylactic          | <b>R</b>                     |       |
| EGFR                               | 10,12   | IL-2                         | 14    | acid                                   | RhIGFBP-3                    | 10    |
| EGFR tyrosine kinase               | 11      | INDO                         | 15    | mPEG-PLA                               | Rosiglitazone XR             | 8     |
| Epidermal growth factor            |         | Indoleamine-pyrrole 2,3-     |       | <b>N</b>                               | <b>S</b>                     |       |
| receptor                           | 5,10,12 | dioxygenase                  | 15    | <i>N</i> -(2-hydroxypropyl)methacryla- | SAHA                         | 15    |
| Erbitux                            | 10      | INMS-18                      | 10    | mide                                   | Secreted phosphoprotein 1    | 5     |
| <i>ERCC5</i>                       | 14      | Insulin-degrading enzyme     | 7     | NEP                                    | Semapimod                    | 14    |
| Erlotinib                          | 10      | Insulin-like growth factor-1 | 10    | Neprilysin                             | SOD1                         | 6     |
| <i>Excision repair cross-</i>      |         | Insulin-like growth factor-1 |       | <b>O</b>                               | <i>SORT1</i>                 | 3,15  |
| <i>complementing rodent repair</i> |         | receptor                     | 12    | <i>Oct4</i>                            | <i>Sortilin 1</i>            | 3,15  |
| <i>deficiency, complementation</i> |         | Insulin-like growth factor   |       | OSI-906                                | Sox2                         | 16    |
| <i>group 5</i>                     | 14      | binding protein-3            | 10,12 | Osteopontin                            | SP-304                       | 13    |
| <b>F</b>                           |         | Insulin receptor substrate-1 | 10    |  | Superoxide dismutase 1       | 6     |
| Folic acid                         | 18      | Iressa                       | 10,12 | <b>P</b>                               | <b>T</b>                     |       |
| Fumagillin                         | 13      | IRS-1                        | 10    | p110 $\beta$                           | Tarceva                      | 5,10  |
| <b>G</b>                           |         | <b>J</b>                     |       | PAI-1                                  | Tissue plasminogen activator | 8     |
| $\gamma$ -LC                       | 16      | JX-594                       | 10    | Panitumumab                            | TNP-470                      | 13    |
| $\gamma$ -secretase                | 9       | <b>K</b>                     |       | PAZ-417                                | tPA                          | 8     |
| $\gamma$ -light chain proteins     | 16      | <i>Klf4</i>                  | 16    | Peroxisome proliferation               | Trastuzumab                  | 11    |
| G-quadruplex motif                 | 17      | <b>L</b>                     |       | activated receptor- $\gamma$           | <b>V</b>                     |       |
| Gefitinib                          | 10,12   | <i>Lactb</i>                 | 14    | Phosphoinositide 3-kinase              | Valproic acid                | 16    |
| Guanylhydrazone                    | 14      | LDL                          | 3     | Phosphoinositide 3-kinase,             | Vectibix                     | 11    |
| Guanylyl cyclase 2C                | 13      | <i>Lipoprotein lipase</i>    | 3,14  | catalytic, $\beta$ -peptide            | VEGF receptor 1              | 6     |
| GUCY2C                             | 13      | Liver X receptor             | 8     | PI3K                                   | VEGF receptor 2              | 6     |
| <b>H</b>                           |         | Lodamin                      | 13    | PI3K- p110 $\beta$                     | VEGF receptor 3              | 6     |
| Hairy-related 2                    | 10      | Low-density lipoprotein      | 3     | PIK3CB                                 | VPA                          | 16    |
| HDAC                               | 15,16   | <i>Lpl</i>                   | 3,14  | Plasmin                                | <b>X</b>                     |       |
| Heat-stable enterotoxin            |         | LXR                          | 8     | Plasminogen                            | XL652                        | 8     |
| receptor                           | 13      | LXR-623                      | 8     | Plasminogen activator                  | <b>Z</b>                     |       |
| HER2                               | 10      | <b>M</b>                     |       | inhibitor-1                            | Zolinza                      | 15    |
| Herceptin                          | 11      | m-THPC                       | 18    | Ploglitazone                           |                              |       |
| Histone deacetylase                | 15,16   | MD-1100                      | 13    | Poliovirus                             |                              |       |
| HPLA                               | 13      |                              |       | Polyethylene glycol                    |                              |       |
|                                    |         |                              |       | PPAR- $\gamma$                         |                              |       |
|                                    |         |                              |       | Ppm1-like                              |                              |       |
|                                    |         |                              |       |  |                              |       |