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By Michael J. Haas, Senior Writer

Less than a year after reporting preclinical proof of concept for a systemically delivered mucopolysaccharidosis IIIA gene therapy, researchers at the **Autonomous University of Barcelona** have shown that intracerebral delivery can substantially improve efficacy in the CNS and treat symptoms in peripheral organs.¹ **Esteve S.A.**, who partially funded the work, is now raising funds to test the approach in the clinic.

Mucopolysaccharidosis IIIA (MPS IIIA)—also called Sanfilippo type A syndrome—is caused by a mutation in *N-sulfoglucosaminase sulfohydrolase (SGSH)* that impairs the ability of SGSH to degrade heparan sulfate glycosaminoglycans (HSGAGs).

The resulting accumulation of HSGAGs in the brain causes a range of motor, behavioral and other neurological symptoms, whereas accumulation in the periphery can lead to enlargement of the liver and spleen. There are no therapies approved to treat the disease, and most patients with MPS IIIA die in their mid-teens.

Therapies in development to treat MPS IIIA include enzyme replacement therapy with SGSH, administered either systemically by i.v. injection or intrathecally by a surgically implanted pump.

Both delivery methods have drawbacks. Systemically administered enzyme cannot penetrate the blood brain barrier (BBB) to treat CNS symptoms. Permanent intrathecal implants can malfunction and induce infections at the implantation site.

Autonomous University of Barcelona (UAB) and Esteve have been exploring alternative approaches. The group focused on adeno-associated virus serotype 9 (AAV9)-based *SGSH* gene therapy after about a decade of research from multiple groups showing that AAV9 isolated from human tissues² could cross the BBB in both directions.^{3,4}

In 2012, the UAB team reported that systemic administration of an AAV9 vector encoding mouse *Sgsh*-induced expression of the enzyme in the brain and normalized HSGAG levels in peripheral organs.⁵

The problem was that the maximum dose of the therapy resulted in *Sgsh* activity in the brain that was only 10%–12% of normal levels and larger doses would have induced significant liver toxicity, team leader Fátima Bosch told *SciBX*.

For the new study, Bosch's team postulated that smaller doses of the therapy injected directly into cerebrospinal fluid (CSF) would have comparable or greater CNS efficacy than systemic administration while safely treating disease symptoms in peripheral organs.

First, the team administered the AAV9 vector encoding murine and canine *Sgsh* to mouse models for MPS IIIA and normal dogs by injecting

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it into the cisterna magna, a space within the cerebellum that is filled with CSF (intracisternal injection). Compared with empty vector, the therapy increased Sgsh activity in the brain to about 40% of normal levels and decreased HSGAG levels in the spleen, kidneys and other organs. In mice, the therapy decreased behavioral deficits and increased locomotor function and survival compared with empty vector or no treatment.

Importantly, the team achieved significant CNS efficacy at doses up to 20-fold lower than the i.v. doses used in its 2012 study.

In normal dogs, intracisternal and intracerebroventricular injections of the AAV9 vector encoding GFP resulted in comparable distributions of the protein throughout the brain and peripheral organs. This finding suggested administering the therapy via clinically established intracerebroventricular procedures would be just as efficacious as intracisternal delivery, which is not commonly used in clinical practice, the team wrote in its report in *The Journal of Clinical Investigation*.

“This is the first demonstration of the whole-body efficacy for a gene therapy to treat a lysosomal storage disorder,” Bosch said.

Bosch is director of the Center of Animal Biotechnology and Gene Therapy at UAB. Her team included researchers from the **St. John of God Hospital** and **The Children’s Hospital of Philadelphia**. Esteve partially funded the study but did not directly participate in it.

“This is the first demonstration of the whole-body efficacy for a gene therapy to treat a lysosomal storage disorder.”

**—Fátima Bosch,
Autonomous University of
Barcelona**

Surgical strike

Michaël Hocquemiller, senior scientific affairs program manager at **Lysogene**, said, “The main theoretical advantage of intracerebroventricular delivery is that one could treat the disease in the periphery in addition to the CNS.”

He cautioned that intracerebroventricular delivery of gene therapy is still at an exploratory stage and that it is unclear how it compares with the intraparenchymal delivery method that Lysogene’s SAF-301 gene therapy uses.

In May, Lysogene completed a Phase I/II study of SAF-301, an intraparenchymally administered AAV10 vector encoding *SGSH* and *sulfatase modifying factor 1 (SUMF1)*, to treat MPS IIIA.

Karen Aiach, cofounder, chairman and CEO of Lysogene, said that Marc Tardieu, principal investigator of that trial, plans to present safety and efficacy data from the trial at the **European Society of Gene and Cell Therapy** conference this October.

Tardieu is professor of pediatrics and head of the pediatric neurology unit at the **University Hospital of Bicêtre**.

The company will also report the trial results in a forthcoming paper, Aiach said.

Intraparenchymal delivery involves drilling six burr holes in the skull and injecting a therapy directly into the brain matter at two different depths in each hole. Intracerebroventricular delivery involves drilling one burr hole and injecting a therapy into CSF contained in brain ventricles.

Olivier Danos, cofounder and senior advisor at Lysogene, said that

the new study “showed that significant amounts of vector had to be used for injection in the cerebrospinal fluid.”

The equivalent dose of the AAV9-based therapy in children would be “more than 100 times greater than the one we used in our clinical trial of SAF-301,” Danos said. Thus, future clinical application of the team’s therapy would be limited by the ability of GMP manufacturers to produce sufficient vector, he said.

Danos added that the safety of the large doses of vector injected in the CSF would have to be established.

Danos also is SVP of molecular medicine, synthetic biology and gene regulation at **Kadmon Corp. LLC**’s Kadmon Pharmaceuticals LLC unit, associate professor at the **University College London**’s Cancer Institute and research director at the **Centre National de la Recherche Scientifique** (CNRS).

However, Mark Mayhew, director of strategic development at Esteve, said the doses used in the *JCI* study would not present the company with manufacturing difficulties.

Moreover, “the ratio of our proposed dose to the volume of CNS target tissue, which includes the whole brain, cerebellum, brain stem and spinal cord, is not higher than those previously shown to be safe in clinical trials” of Leber’s congenital amaurosis (LCA) and Parkinson’s disease (PD), he said.

Indeed, the vector injected into the CSF distributes evenly across the CNS and periphery, resulting in vector levels that are much lower than those found near intraparenchymal injection sites, he said.

Bosch pointed out that—as reported in the *JCI* paper—her team observed no signs of CNS inflammation or other safety problems in a seven-day follow-on study in normal dogs that received intracisternal injections of AAV9 vector encoding canine *SGSH*.

Additionally, the intracerebroventricular injection is already used to treat hydrocephalus (abnormal accumulation of CSF in brain ventricles) and deliver antibiotics, pain therapies and cancer drugs in 40,000–50,000 pediatric patients in the U.S. each year, giving it a safety track record that intraparenchymal delivery does not yet have, said Eduard Valenti, Esteve’s director of regulatory affairs and pharmaceutical quality.

“Our approach uses a standard procedure that most neurosurgeons have the skills to perform,” Bosch added.

Intraparenchymal delivery requires special expertise and a special injection device that most pediatric surgeons lack, she said.

“Intracerebroventricular delivery is less invasive than intraparenchymal delivery and gets the therapeutic vector into the ventricle through a part of the brain that does not house important functions, so the risk of damage is minimal,” said Virginia Haurigot, a research associate in Bosch’s group at UAB and first author on the *JCI* paper. “Also, direct delivery into CSF allows the therapy to reach parts of the CNS that are hard to reach with intraparenchymal injection.”

Both Haurigot and Mayhew said the efficacy of intraparenchymal gene therapy was not yet established.

Haurigot said that published studies in animals have shown that intraparenchymally delivered vectors have limited diffusion in the CNS. “Therefore you cannot reach very deep structures of the brain

such as the cerebellum and brain stem, and transfection of the brain stem appears to correlate with efficacy” for at least some CNS diseases, such as Canavan disease,⁶ she said.

Mayhew added, “It’s also not clear whether intraparenchymal therapies can cross the blood brain barrier and get into the periphery.”

According to Hocquemiller, at least two studies have shown that i.v. AAV10 crosses the BBB as efficiently as AAV9.^{7,8} Although he had not seen any studies showing that an intraparenchymally delivered vector could cross the BBB, “if that did happen, the amount would probably not be sufficient to treat symptoms in peripheral organs,” he said.

Different development vectors

Hocquemiller and Danos both said that the results of the *JCI* paper raise another obstacle for the AAV9 vector that AAV10 does not have.

“The study shows that the presence of pre-existing serum antibodies against the AAV9 vector is likely to block the vector’s transduction of peripheral organs,” thereby reducing its efficacy in those organs, Hocquemiller said.

In the study, the team injected an AAV9 vector encoding *GFP* into dogs that were preimmunized against AAV9. Whereas the low levels of anti-AAV9 antibodies in CSF did not significantly affect the distribution of *GFP* in the brain, the high levels of serum antibodies did reduce the ability of the vector

to reach peripheral organs and express *GFP*.

“Up to 80% of humans have serum antibodies against AAV9, AAV2 and/or AAV5, which may limit the use of these as vectors for gene therapy,” Danos said. “Lysogene’s AAV10 vector, which is derived from a rhesus monkey, may be more applicable to gene therapy” because antibodies against it have not been found in humans.

Haurigot acknowledged that “some efficacy was lost in the periphery due to serum anti-AAV9 antibodies” but noted that there was no illness or adverse event associated with them.

But because MPS IIIA is primarily a neurodegenerative disease, “the main target of the therapy is the brain, and we still achieved efficacy in the CNS even when the dogs had pre-existing immunity to AAV9,” she said.

Haurigot added that the likelihood of developing anti-AAV9 antibodies increases with age, but young children comprise the target population for MPS IIIA therapies. Thus, “a large proportion of patients may benefit from the peripheral efficacy of our therapy because they would not have been previously exposed to AAV9,” she said.

Going forward, Esteve is seeking investors or corporate partners to help fund a Phase I/II trial of intracerebroventricular AAV9-based *SGSH* gene therapy to treat MPS IIIA. The start date of the trial would depend on when the company secures the necessary funding, Mayhew said.

Mayhew added that **ReGenX Biosciences LLC** owns IP around many AAV vectors, including AAV9. Thus, “the entity that eventually commercializes our gene therapy would have to come to some arrangement with ReGenX,” he said.

Meanwhile, Bosch’s team is testing AAV9-based gene therapy in animal models of MPS IIIB (Sanfilippo type B syndrome), a disease that is caused by a mutation in *N-acetylglucosaminidase- α* (*NAGLU*),

“The study shows that the presence of pre-existing serum antibodies against the AAV9 vector is likely to block the vector’s transduction of peripheral organs.”

—*Michaël Hocquemiller, Lysogene*

and other types of MPS.

UAB and Esteve hold a patent for the AAV9-based gene therapy to treat MPS IIIA, and the IP is exclusively licensed to Esteve.

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COMPANIES AND INSTITUTIONS MENTIONED

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MJFF's progress in markers

By Michael J. Haas, Senior Writer

The Michael J. Fox Foundation for Parkinson's Research has taken two major steps toward its goal of identifying clinical populations in which new therapies for Parkinson's disease can be tested. Last week, the foundation made available the first set of data and samples from its LRRK2 Cohort Consortium and presented preliminary data from its Parkinson's Progression Marker Initiative.

Now, the LRRK2 Cohort Consortium will conduct longitudinal studies in its enrolled participants, whereas the Parkinson's Progression Marker Initiative (PPMI) aims to confirm the preliminary findings in the full study cohort. Additionally, the PPMI is planning collaborations with the Alzheimer's Association's Alzheimer's Disease Neuroimaging Initiative (ADNI).

The LRRK2 story dates to 2004, when two independent teams identified mutations in *leucine-rich repeat kinase 2 (LRRK2)* in patients who had an inherited form of PD. "That discovery galvanized a lot of industry interest because LRRK2 is a protein kinase with gain-of-function mutations in PD, and so it seemed a tractable target for inhibition," Todd Sherer, CEO of MJFF, told *SciBX*.

In parallel with industry efforts to develop LRRK2 inhibitors, MJFF sought "to get in front of the curve by developing a cohort of individuals with *LRRK2* mutations that would help establish the infrastructure for later clinical and biomarker research," he said.

Thus, MJFF launched the LRRK2 Cohort Consortium in 2009 with a pilot program that included two populations—Ashkenazi Jews and North African Berbers—in whom *LRRK2* mutations account for about 20% and 40% of PD cases, respectively.

"We brought together the researchers at centers in New York, Tel Aviv and Tunisia with a shared core protocol" for cross-sectional studies in patients with PD, healthy individuals who harbored the *LRRK2* mutations and healthy individuals who did not, Sherer said.

In 2011, MJFF expanded the consortium to include a total of about 1,900 individuals at clinical centers in 9 countries, including Norway, Spain, Germany and Canada.

Sherer said the purpose of the cross-sectional studies was twofold. One goal was to compare LRRK2 PD and idiopathic PD. The other was to pre-recruit a population of individuals carrying *LRRK2* mutations for future clinical trials, "so that the trials don't have to start recruiting from scratch and there will be some run-in data for the population," he said.

Now that the consortium has completed those cross-sectional studies, MJFF has made the clinical data and biological samples—including whole blood, serum, urine and cerebrospinal fluid (CSF)—available to academic and industry researchers for the first time.

Although any qualified researcher can gain access to the clinical data,

"we will set a higher bar of acceptance for proposed projects that would use the biological samples because they are a limited resource," Sherer said. For example, "we would want to see data to confirm the reliability and validity of an assay that would use the samples."

He added, "We are especially interested in projects involving pharmacodynamic measurements of LRRK2 levels and activity in the samples that could be used to measure outcomes in trials of LRRK2-related therapies."

PD researchers in academia and industry can apply for access to the consortium's data and specimens by submitting project proposals. MJFF will review new proposals and decide whether to accept them only in August, October and December.

"We set the deadlines to encourage researchers to apply in a timely manner," Sherer said.

Academic and industry researchers can also apply to MJFF for up to \$250,000 in funding for projects that use data or samples from the LRRK2 Cohort Consortium.

Meanwhile, all but one of the groups within the consortium is converting the cross-sectional studies to longitudinal studies. "The Tunisian group is not lined up to continue due to the geopolitical situation in the country," Sherer said. "It's not clear to us how we could continue that study—but this doesn't mean it will never continue."

MJFF expects to make preliminary data from the longitudinal studies available in 2014 "rather than waiting for the studies to finish in late 2018 or early 2019."

To date MJFF has invested more than \$10 million in the LRRK2 Cohort Consortium. The clinical centers have also invested in the consortium.

"We see the LRRK2 Cohort Consortium as an example of the exact role the foundation can play because the findings are a resource that

everyone in industry is looking for but will not necessarily have funded in house," Sherer said.

"We see the LRRK2 Cohort Consortium as an example of the exact role the foundation can play because the findings are a resource that everyone in industry is looking for but will not necessarily have funded in house."

— Todd Sherer,

The Michael J. Fox Foundation for Parkinson's Research

PD and AD overlap

MJFF also announced the availability of data and samples from its PPMI, a five-year program launched in 2010 to identify biological markers for PD.

The program initially enrolled about 150 patients and age-matched controls in the U.S. and EU¹ and since has expanded to include about 600 patients and controls at 24 sites in the U.S., EU and Australia.

According to Mark Frasier, MJFF's VP of research programs, the PPMI program is examining the levels of four proteins in CSF: α -synuclein (SNCA), β -amyloid (A β), microtubule-associated protein- τ (MAPT; TAU; FTDP-17) and phosphorylated- τ (p- τ).

"In the preliminary data from the first 100 participants who enrolled in PPMI, we've found that the levels of all four proteins are lower in the CSF of PD patients than in the healthy controls," Frasier said.

Additionally, the preliminary data showed differences in the levels of the markers between subgroups of patients with PD whose clinical symptoms differ, he said. "For example, in patients who experience significant problems with posture and gait, the levels of all four markers

are lower than in patients who experience significant problems with tremors."

These findings suggest the CSF markers could be used to stratify patients in clinical trials of therapies to treat symptoms associated with particular subtypes of PD.

Frasier said PPMI researchers also compared the preliminary data with those from the Alzheimer Association's ADNI program, which is examining the same four CSF markers in patients with AD and healthy controls, and found some interesting overlaps.

Indeed, MJFF's interest in markers extends beyond LRRK2 and even beyond PD itself.

For example, in patients with AD, A β is also lower but TAU is higher than levels in controls, he said. "The findings point to a potential role for TAU in both PD and AD, though we don't yet know TAU's role in memory impairment in AD or its role in PD symptoms."

Kenneth Marek presented the preliminary PPMI data and the comparisons with the ADNI results at the Alzheimer's Association International Conference last week. Marek is a clinical professor of neurology at **Yale University** and president and senior scientist at the university's Institute for Neurodegenerative Disorders.

Frasier said a paper reporting the preliminary data is in the press.

Going forward, PPMI researchers will analyze the markers in the full cohort of 600 patients with PD and controls to confirm the preliminary findings, Frasier said.

"Also, we will examine how these markers change over time by comparing samples taken at six months and one year with the baseline

samples and see how levels of the markers might change as the disease progresses," he said.

Frasier added that PPMI and ADNI plan to use data from both programs to investigate questions about aging and neurodegeneration and want to add new parameters or studies to each program.

"There is momentum among researchers in both PD and AD to test drugs earlier in the disease process—before overt symptoms appear—because we think the greatest chance for therapeutic success occurs when the loss of brain cells is minimal," which, in turn, underscores the need to identify the right population and methods for clinical trials, he said.

The markers studied in the PPMI and ADNI programs "could help identify the presymptomatic population for testing new therapies in the clinic," said Frasier. "Both Parkinson's disease and Alzheimer's disease research is moving in this direction."

MJFF plans to disclose details about the PPMI-ADNI collaborations in the coming months. To date MJFF has invested \$55 million in the PPMI program.

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Silencing neuropathic pain

By C. Simone Fishburn, Senior Editor

The seemingly intractable nature of neuropathic pain has led drug developers to look beyond classical receptor and transporter protein targets and explore the emerging field of epigenetics to find new options. Now, researchers at **The Johns Hopkins University School of Medicine** have found a potential target based on the discovery that nerve injury upregulates a long noncoding RNA that silences voltage-gated potassium channel Kv1.2 exclusively in damaged neurons.¹

Neuropathic pain develops after nerves are damaged by injury, viral infection or disease, which set up the nervous system's controls to go awry. Under these conditions, neurons spontaneously fire signals to the brain, sending pain messages even when there is no physical stimulus.

Yuan-Xiang Tao, an associate professor of anesthesiology and critical care medicine at The Johns Hopkins University School of Medicine, told *SciBX* that his team began looking for epigenetic causes of the condition five years ago as data emerged on the importance of microRNA and noncoding RNA in diseases of the nervous system.²

In 2009, the group performed proteomic studies to explore how nerve injury alters gene expression in the dorsal root ganglia (DRG),³ which are nodules that house the cell bodies of peripheral nerves. Now, the team has honed in on noncoding RNAs and discovered a 2.5 kb long noncoding RNA (lncRNA) that is upregulated in damaged neurons.

Interestingly, the lncRNA sequence was overlapping with and complementary to the coding region of the voltage-gated potassium channel *Kv1.2* (*KCNA2*), suggesting it could decrease the expression of *KCNA2* and affect the axonal membrane potential, leading to increased neuronal firing (see **Figure 1**, "Making antisense of *Kcna2* in pain").

KCNA2 is expressed in the brain and spinal cord. It is a potential therapeutic target for epilepsy because of the high seizure rate observed in *Kcna2*^{-/-} knockout mice.⁴

The Johns Hopkins team showed that *Kcna2* lncRNA reproduced symptoms of neuralgia in rats when it was overexpressed in DRG neurons. Conversely, *Kcna2* mRNA alleviated symptoms of neuralgia in rat models for neuropathic pain when delivered to the DRG.

Furthermore, the upregulation of *Kcna2* lncRNA that occurred following nerve injury was accompanied by a decrease in *Kcna2* mRNA and protein levels in individual DRG neurons. That finding suggests *Kcna2* lncRNA may act at least in part by directly neutralizing *Kcna2* mRNA.

Data were reported in *Nature Neuroscience*.

According to Jim Barsoum, CSO at epigenetics drug discovery company **RaNA Therapeutics Inc.**, one of the most interesting aspects of the study is that the lncRNA is upregulated in response to nerve damage.

Although antisense lncRNAs are commonly expressed at considerably lower levels than their corresponding sense RNAs, and their upregulation has been associated with several chronic diseases and genetic disorders, it is rare to see such a rapid and significant increase in an lncRNA caused by injury.

Barsoum thinks the antisense lncRNA likely works not only by

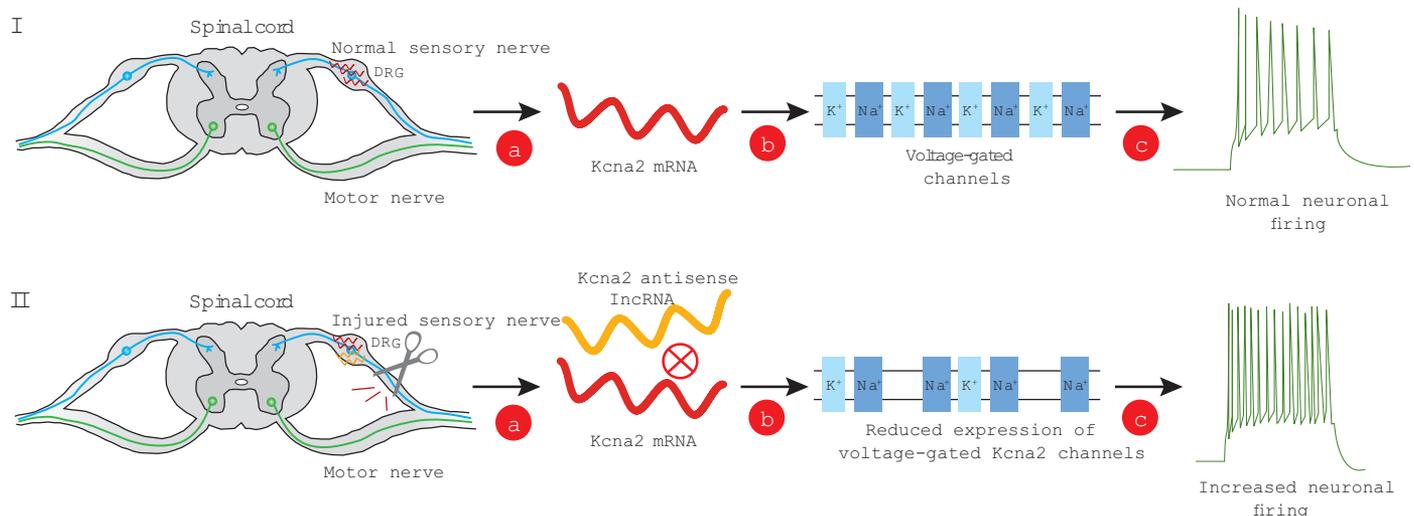


Figure 1. Making antisense of *Kcna2* in pain. Pain sensation involves transmission of signals by sensory nerves through the spinal cord to motor nerves, which control the physiological motor response.

(I) In normal neurons, *potassium channel Kv1.2* (*Kcna2*) mRNA [a] is produced in sensory nerve cell bodies that lie in the dorsal root ganglia (DRG), leading to expression of *Kcna2* voltage-gated potassium channels [b]. The resulting potassium channel conductance counterbalances the sodium influx, stabilizing the neuronal membrane and enabling normal neuronal firing [c].

(II) In injured nerves, antisense *Kcna2* lncRNA is upregulated [a] and silences normal expression of *Kcna2* voltage-gated potassium channels [b]. The reduced potassium conductance alters the balance of ion fluxes, which decreases the threshold for action potential firing compared with that in uninjured nerves and creates hyperexcitable neurons [c].

blocking the sense strand but also by recruiting other epigenetic modulators that control the transcription of the channel.

Sensing a therapy

Turning the Johns Hopkins findings into a therapeutic would likely involve inactivating or eliminating the increase in *KCNA2* lncRNA.

Barsoum thinks this would most likely require using small interfering RNA or a single-stranded oligonucleotide to block the interaction between the antisense and sense transcripts or cause degradation of the lncRNA.

According to Barsoum, single-stranded oligonucleotides might represent the best option, as they have been successfully delivered to several tissues including neuronal cells.^{5,6} They display long half-lives inside the CNS and thus might have an effect that lasts long enough to avoid the need for frequent dosing.

By contrast, double-stranded oligonucleotides require lipid-based nanoparticle formulations that often have toxicity problems.⁷ Additionally, strategies to boost sense RNA would be complicated by the need to deliver the precise dose for sufficient—but not excess—expression of *KCNA2* channels.

Charles Cohen, VP of biological sciences at clinical genetics company **Xenon Pharmaceuticals Inc.**, said a product targeting *KCNA2* lncRNA would most likely need to be delivered intrathecally to achieve sufficient concentrations in the DRG. Cohen previously developed compounds for neuropathic pain at **Vertex Pharmaceuticals Inc.**

Cohen said intrathecal delivery has been employed successfully in treating pain, citing Prialt ziconotide (SNX-111) from **Jazz Pharmaceuticals plc**. He did note that a narrow therapeutic index limits more widespread use of the drug and that a *KCNA2* lncRNA-targeted therapy might need to have a better safety profile than ziconotide to be competitive.

Cohen also said the widespread distribution of *KCNA2* channels

throughout the brain could give rise to side effects and noted that preclinical studies can be misleading because of significant differences in distribution between rodents and primates.

However, the *Kcna2* lncRNA transcript was only upregulated in damaged neurons, and no changes were observed in uninjured DRG neurons. Thus, it is possible that a therapy targeting *KCNA2* lncRNA would not affect *KCNA2* activity in normal cells.

At least four companies have preclinical programs involving lncRNAs. These include RaNA; **Isis Pharmaceuticals Inc.**; Opko-Curna, a unit of **Opko Health Inc.**; and **TransSINE Technologies Co. Ltd.**

The findings of the study have not been patented. The authors said they are exploring collaborations for clinical studies.

Fishburn, C.S. *SciBX* 6(28); doi:10.1038/scibx.2013.711
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Jazz Pharmaceuticals plc (NASDAQ:JAZZ), Dublin, Ireland
The Johns Hopkins University School of Medicine, Baltimore, Md.
Opko Health Inc. (NYSE:OPK), Miami, Fla.
RaNA Therapeutics Inc., Cambridge, Mass.
TransSINE Technologies Co. Ltd., Yokohama, Japan
Vertex Pharmaceuticals Inc. (NASDAQ:VRTX), Cambridge, Mass.
Xenon Pharmaceuticals Inc., Burnaby, British Columbia, Canada

Universal appeal for CARs

By Tracey Baas, Senior Editor

Chimeric antigen receptor-expressing T cells have shown dramatic efficacy in treating blood cancers, but so far only autologous, modified T cells can be used, making manufacturing labor intensive and expensive. Now, **The University of Texas MD Anderson Cancer Center** and **Sangamo BioSciences Inc.** have taken a step toward a universal immunotherapy by using gene-modifying tools to mask donor-derived T cells from the standard immune surveillance machinery in the recipient.¹ The MD Anderson team is already planning clinical trials with such cells targeting CD19.

Universal, chimeric antigen receptor (CAR)-expressing T cells would have two key traits—the cells would not attack the host via the graft-versus-host (GvH) response and the host would not attack the cells via the host-versus-graft (HvG) response.

GvH is mediated by T cell receptors (TCRs) on the infused allogeneic cells that are stimulated by mismatched human leukocyte antigens (HLAs) on the recipient's normal tissues. HvG is mediated by host-derived T cells that employ TCRs to recognize mismatched HLAs on infused allogeneic cells.

Mismatches occur because each individual presents a unique combination of HLA class I antigens and HLA class II antigens. *HLA* alleles are highly polymorphic, and finding an appropriately matched, unrelated, universal donor for multiple recipients would be unattainable.

With allogeneic bone marrow transplantation, optimal outcomes for unrelated donors occur when a minimum of class I antigens major histocompatibility complex class I A (HLA-A), HLA-B and HLA-C and class II antigen major histocompatibility complex class II DR (HLA-DR) have been matched between one donor and the intended recipient.²

What constitutes minimally matched T cells for cancer patient recipients is unknown. Therefore, genetic strategies have been developed to eliminate HLA expression on donor T cells.

In the new study, a team of researchers at MD Anderson co-led by Laurence Cooper and Hiroki Torikai collaborated with Sangamo to investigate whether an immune response to allogeneic, CAR-expressing T cells could be avoided by eliminating expression of one or more mismatched HLAs on donor-derived cells.

Cooper is a professor of pediatrics, section chief of cell therapy for the Children's Cancer Hospital and associate director of the Center for Cancer Immunology Research at MD Anderson, and Torikai is a research scientist at MD Anderson.

The team had previously used zinc finger nucleases (ZFNs) to eliminate expression of TCRs on human CAR-expressing T cells specific for CD19. *In vitro*, the ZFN-edited, TCR⁻ cells retained their antitumor activity against malignant B cells and did not respond to TCR stimulation, which is the initiating factor for a GvH response.³

Based on the success using ZFNs to control the GvH response, the group hypothesized that it could also control HvG by eliminating HLAs to generate universal, CAR-expressing T cells.

As proof of concept, the team designed and used ZFNs to eliminate expression of *HLA-A2* and *HLA-A3* in an immortalized human cell line. Isolated cellular clones lacking the *HLA*-As were not recognized or lysed by *HLA-A2*- and *HLA-A3*-restricted cytotoxic T lymphocytes (CTLs).

The team then generated *HLA-A*⁻ primary T cells via a two-step procedure. First, the team used electroporation to deliver ZFN mRNA into T cells to eliminate *HLA-A2*, which is the most common *HLA-A* allele in Caucasians. The researchers then used antibody-coated paramagnetic beads to remove unedited *HLA-A2*⁺ T cells in which the ZFNs did not cleave *HLA-A2*. The result was a population of T cells in which more than 95% were *HLA-A2*⁻.

With proof of concept in hand for the procedure, the researchers turned their attention to modifying CD19-specific, CAR-expressing T cells. Using ZFN mRNA electroporation and paramagnetic bead selection, the team generated a population of *HLA-A2*⁻, CD19-specific, CAR⁺ T cells with around 99% enrichment.

Even after multiple days in culture, about 94% of the CD19-specific, CAR-expressing T cells remained *HLA-A2*⁻ and avoided attack by *HLA-A2*-restricted CTLs, suggesting the HvG response had been thwarted. Additionally, the modified T cells retained their ability to lyse CD19⁺ lymphoma cell lines as well as CD19⁺ primary lymphoma cells obtained from patients, indicating that

the cells maintained their antitumor activity.

Next, the team simultaneously eliminated *HLA-A2* and TCR in primary T cells by using two ZFN species. The result was elimination of both GvH and HvG.

Finally, the group attempted to protect the T cells from another type of HvG response that is initiated by NK cells. T cells lacking classical HLA class I molecules, such as *HLA-A*, are seen as nonself and are subject to elimination by NK cells.

The team simultaneously expressed nonclassical class I antigens *HLA-E* and *HLA-G* on *HLA-A*⁻ cells and showed that those expressing either one or both of the molecules were lysed significantly less often by NK cells than those lacking all class I molecules.

Results were published in *Blood*.

Stepwise into the clinic

The MD Anderson team is planning clinical trials of CD19-specific, CAR-expressing T cells using an escalating approach—first TCR⁻ cells and then TCR⁻ and *HLA*⁻ cells. Initially, TCR⁻ T cells will be tested in humans. An application has been submitted to the **NIH** Office of Biotechnology Activities seeking federal regulatory approval.

“Our first trial will infuse CD19-specific CAR T cells that have been genetically edited to eliminate the expression of endogenous TCR,” said Cooper. “These trials will then be modified to infuse CD19-specific CAR T cells that lack TCR as well as *HLA-A* to improve persistence and to avoid presentation of immunogenic genes.”

“To edit genes, we are working with Sangamo's designer ZFNs. They

“What range of HLA class I or HLA class II molecules will need to be targeted, in conjunction with TCR editing, to provide the best therapeutic cells is hard to predict.”

—Carl June,
Perelman School of Medicine at the
University of Pennsylvania

“For truly universal cells, all classical HLA I and HLA II molecules would need to be eliminated to prevent host-versus-graft reactions, while TCR would need to be eliminated to prevent graft-versus-host reactions.”

—Zelig Eshhar,
Weizmann Institute of Science

that is not limited to ZFNs but include CRISPR [clustered, regularly interspaced short palindromic repeats] or TALEN [transcription activator–like effector nuclease] gene editing systems,” added Cooper.

“Universal therapy using HLA-edited allogeneic T cells is absolutely desirable because banking HLA-matched cell lines would not cover all HLA matching options, and even more importantly, the outcome of the therapy would be very heterogeneous depending on the exact level of matching,” said Assaf Marcus, a postdoctoral researcher in the Department of Molecular and Cell Biology at the **University of California, Berkeley**.

Marcus and Zelig Eshhar, a professor of chemical and cellular immunology at the **Weizmann Institute of Science**, both think the CARs will require additional modifications before they can become off-the-shelf products.

“HLA-A⁻ cells are not universal cells; they are proof of concept that T cells can be made more stealth-like to CTLs,” said Eshhar. “For truly universal cells, all classical HLA I and HLA II molecules would need to be eliminated to prevent host-versus-graft reactions, while TCR would need to be eliminated to prevent graft-versus-host reactions.”

“And because NK cells can rapidly eliminate massive numbers of donor cells that lack HLA I expression, HLA-E and/or HLA-G expression would most likely be required to give donor cells a fighting chance to outmaneuver the recipient’s immune system and establish donor cell persistence,” said Eshhar.

“If one could ask to see the best next steps, that would be to generate allogeneic T cells lacking all classical HLA I and II molecules and TCR and expressing HLA-E and HLA-G,” proposed Bruce Levine, an associate professor in cancer gene therapy in the Department of Pathology and Laboratory Medicine at the **Abramson Cancer Center at the University of Pennsylvania**. “But just because you can edit any or all those proteins doesn’t make it necessary and doesn’t make it practical.”

Carl June, a professor in the Department of Pathology and Laboratory Medicine at the **Perelman School of Medicine at the University of Pennsylvania** and director of the translational research program at

have terrific experience supporting the human application of T cells edited with ZFNs. In the future, clinical-grade T cells, as well as other cells, will be genetically edited to eliminate undesired genes. These can be generated using a panel of artificial nucleases

Abramson, thought the MD Anderson and Sangamo concept is worth probing in the clinic.

June’s three-patient Phase I trial using CD19 CAR T cells in chronic lymphocytic leukemia (CLL) basically set the field ablaze^{4,5} and led to a deal in 2012 between the **University of Pennsylvania** and **Novartis AG** to develop and commercialize CAR immunotherapies for cancer.⁶

“What range of HLA class I or HLA class II molecules will need to be targeted, in conjunction with TCR editing, to provide the best therapeutic cells is hard to predict,” June said.

He said the planned escalation approach is a good idea. “Sequentially testing increasingly modified cells to determine safety and persistence” is a sound strategy, said June. “It is hard to overestimate the ability of the human immune system to detect and eliminate modified cells.”

“These types of studies will likely uncover modified allogeneic T cells that could be used as universal, CAR-based therapies for tumor knockdown or remission induction and therapies to prevent tumor relapse,” he continued. “However, I am doubtful that these universal cells will have long-term persistence and therefore may not be a substitute for autologous T cells.”

The University of Texas MD Anderson Cancer Center Office of Technology Commercialization and Sangamo have jointly filed for a patent covering universal CD19-specific, CAR-expressing T cells. The IP is available for licensing.

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University of California, Berkeley, Calif.
University of Pennsylvania, Philadelphia, Pa.
The University of Texas MD Anderson Cancer Center, Houston, Texas
Weizmann Institute of Science, Rehovot, Israel

This week in therapeutics

THE DISTILLERY brings you this week's most essential scientific findings in therapeutics, distilled by *SciBX* editors from a weekly review of more than 400 papers in 41 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

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

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Autoimmune disease				
Autoimmune disease	Calcium channel N-type; calcium channel P/Q-type	Cell-based and mouse studies identified an agonist of N- and P/Q-type calcium channels that could help treat Lambert-Eaton myasthenic syndrome. In cultured cells expressing N- or P/Q-type calcium channels, the roscovitine derivative GV-58 increased calcium conductance compared with vehicle control. In a mouse model for Lambert-Eaton myasthenic syndrome, GV-58 increased transmitter release from neuromuscular junctions compared with vehicle. Next steps include optimizing the compound and conducting tests in additional models for neuromuscular disorders.	Patent application filed; available for licensing	Tarr, T.B. <i>et al. J. Neurosci.</i> ; published online June 19, 2013; doi:10.1523/JNEUROSCI.4629-12.2013 Contact: Stephen D. Meriney, University of Pittsburgh, Pittsburgh, Pa. e-mail: meriney@pitt.edu Contact: Peter Wipf, same affiliation as above e-mail: pwipf@pitt.edu
SciBX 6(28); doi:10.1038/scibx.2013.713 Published online July 25, 2013				
Cancer				
Cancer	Ataxia telangiectasia mutated (ATM); DNA-dependent protein kinase (DNA-PK)	Mouse and cell culture studies suggest inhibiting the DNA-PK catalytic subunit could help treat ATM-deficient cancers. In ATM-deficient human lung cancer cell lines, small hairpin RNA-mediated knockdown of the DNA-PK catalytic subunit decreased growth compared with no knockdown. In a mouse model for non-Hodgkin's lymphoma (NHL), shRNA-mediated knockdown of Atm plus a small molecule inhibitor of the DNA-PK catalytic subunit increased survival compared with no treatment. Celgene Corp's CC-115, a dual inhibitor of DNA-PK and mammalian target of rapamycin (mTOR; FRAP; RAFT1), is in Phase I testing to treat multiple myeloma, NHL and solid tumors.	Patent application filed covering use in NHL; unavailable for licensing	Riabinska, A. <i>et al. Sci. Transl. Med.</i> ; published online June 12, 2013; doi:10.1126/scitranslmed.3005814 Contact: Hans Christian Reinhardt, University Hospital of Cologne, Cologne, Germany e-mail: christian.reinhardt@uk-koeln.de Contact: Shuhua Chen, same affiliation as above e-mail: shuhua.chen@uni-koeln.de
SciBX 6(28); doi:10.1038/scibx.2013.714 Published online July 25, 2013				
Cancer	Proteasome	Cell culture studies suggest a noncompetitive, imidazoline-based proteasome inhibitor could help treat cancer. In <i>in vitro</i> assays, the lead compound inhibited the proteasome with an IC ₅₀ value of 130 nM without targeting the proteasome catalytic site. In cultured human myeloma cell lines sensitive or insensitive to Velcade bortezomib, the lead imidazoline-based inhibitor caused greater cell death than the parent inhibitor. Next steps include evaluating the <i>in vivo</i> toxicity of the lead inhibitor. Takeda Pharmaceutical Co. Ltd. and Johnson & Johnson market the competitive proteasome inhibitor Velcade to treat multiple myeloma (MM) and mantle cell lymphoma. Onyx Pharmaceuticals Inc. and Ono Pharmaceutical Co. Ltd. market the selective proteasome inhibitor Kyprolis carfilzomib to treat MM. At least 10 companies have proteasome inhibitors in Phase III testing or earlier development to treat cancer.	Patented; available for licensing from Michigan State University Technologies	Azevedo, L.M. <i>et al. J. Med. Chem.</i> ; published online June 22, 2013; doi:10.1021/jm400235r Contact: Jetze J. Tepe, Michigan State University, East Lansing, Mich. e-mail: tepe@chemistry.msu.edu
SciBX 6(28); doi:10.1038/scibx.2013.715 Published online July 25, 2013				

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Gastrointestinal cancer	Stem cell factor receptor tyrosine kinase (c-Kit; KIT; CD117)	<p><i>In vitro</i> studies suggest quinazoline-pyrazolourea-based KIT inhibitors could help treat Gleevec imatinib-resistant gastrointestinal stromal tumors (GISTs). Chemical synthesis, SAR and <i>in vitro</i> testing identified quinazoline-pyrazolourea analogs that inhibited wild-type KIT and two imatinib-resistant KIT mutants with low nanomolar IC₅₀ values. In human GIST cell lines expressing constitutively active KIT or the imatinib-resistant T670I KIT mutant, 3 of the lead compounds caused 50% growth inhibition at low nanomolar concentrations. Future studies could include testing the lead compounds in animal models bearing imatinib-resistant GIST xenografts.</p> <p>Novartis AG markets Gleevec, a BCR-ABL tyrosine kinase inhibitor, to treat GISTs, acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML) and other hematological malignancies. The drug also is under review for hypertension and in Phase II testing to treat melanoma and scleroderma.</p> <p>AB Science S.A.'s KIT inhibitor masitinib is under review for imatinib-resistant GISTs and in Phase III testing to treat other cancers.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.716 Published online July 25, 2013</p>	Patent and licensing status unavailable	<p>Richters, A. <i>et al. J. Med. Chem.</i>; published online June 17, 2013; doi:10.1021/jm4004076</p> <p>Contact: Daniel Rauh, Technical University of Dortmund, Dortmund, Germany e-mail: daniel.rauh@tu-dortmund.de</p> <p>Contact: Sebastian Bauer, University of Duisburg-Essen Medical School, Essen, Germany e-mail: sebastian.bauer@uk-essen.de</p>
Melanoma	E2F1; tumor protein p73 (TP73; p73)	<p>Cell culture and mouse studies suggest combining methotrexate with an antifolate prodrug could help treat melanoma. In a panel of human melanoma cell lines with different oncogenic mutations, including lines with acquired resistance to BRAF and MEK inhibitors, methotrexate plus the antifolate prodrug 3-O-(3,4,5-trimethoxybenzoyl)-(-)-epicatechin (TMECG) upregulated E2F1 and p73 and decreased growth compared with either compound alone. In a mouse xenograft model for melanoma, the combination decreased tumor growth compared with either treatment alone. Next steps include conducting pharmacokinetic and toxicology studies of TMECG.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.717 Published online July 25, 2013</p>	Patent application filed; available for licensing	<p>Saéz-Ayala, M. <i>et al. Cancer Cell</i>; published online June 20, 2013; doi:10.1016/j.ccr.2013.05.009</p> <p>Contact: José Neptuno Rodríguez-López, University of Murcia, Murcia, Spain e-mail: neptuno@um.es</p> <p>Contact: Colin R. Goding, University of Oxford, Oxford, U.K. e-mail: colin.goding@ludwig.ox.ac.uk</p>
Melanoma	Mitochondrial oxidative phosphorylation	<p>Cell culture and mouse studies suggest inhibiting oxidative phosphorylation could help treat drug-resistant melanoma. In cultured, drug-resistant melanoma cells that highly expressed jumonji AT rich interactive domain 1B (JARID1B; PLU-1), multiple proteins involved in oxidative phosphorylation were overexpressed compared with expression in control cells. A series of compounds that inhibit mitochondrial ATP production increased cell death compared with vehicle. In xenograft mouse models for melanoma, combining inhibitors of oxidative phosphorylation with anticancer drugs decreased tumor growth compared with either set of compounds alone. Next steps include understanding the basis for mitochondria-mediated drug resistance in JARID1B-overexpressing cells and could include a clinical trial of Zelboraf vemurafenib plus phenformin in melanoma.</p> <p>Roche, Daiichi Sankyo Co. Ltd. and Chugai Pharmaceutical Co. Ltd. market Zelboraf vemurafenib, a small molecule inhibitor of BRAF V600E, for melanoma. Phenformin, a biguanide that inhibits oxidative phosphorylation, is a generic diabetes drug.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.718 Published online July 25, 2013</p>	Patent and licensing status undisclosed	<p>Roesch, A. <i>et al. Cancer Cell</i>; published online June 10, 2013; doi:10.1016/j.ccr.2013.05.003</p> <p>Contact: Meenhard Herlyn, The Wistar Institute, Philadelphia, Pa. e-mail: herlynm@wistar.org</p> <p>Contact: Alexander Roesch, Saarland University Hospital, Homburg, Germany e-mail: alexander.roesch@uks.eu</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Melanoma	Peroxiredoxin 2 (PRDX2)	<p>Mouse studies suggest compounds that mimic PRDX2 activity could help prevent melanoma metastasis. In mice, melanoma cells pretreated with Prx2-targeting small interfering RNA showed greater lung metastasis than cells pretreated with control siRNA. In the same model, mice treated with gliotoxin, a fungal metabolite with peroxiredoxin-like activity, had lower lung metastasis than mice given vehicle. Next steps include testing the compound's efficacy in a genetic mouse model more relevant to metastatic melanoma in humans.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.719 Published online July 25, 2013</p>	Patent application filed; available for licensing through the Ewha University-Industry Collaboration Foundation	<p>Lee, D.J. <i>et al. Cancer Res.</i>; published online June 7, 2013; doi:10.1158/0008-5472.CAN-12-4226 Contact: Sang Won Kang, Ewha Womans University, Seoul, South Korea e-mail: kangsw@ewha.ac.kr</p>
Neuroendocrine tumors	Aurora kinase A (AURKA; aurora-A); v-myc myelocytomatosis viral related oncogene neuroblastoma derived (MYCN; NMYC)	<p>Cell culture and mouse studies suggest AURKA inhibitors that disrupt the AURKA-NMYC complex could help treat neuroblastomas. In NMYC-driven neuroblastoma cell lines, AURKA inhibitors decreased NMYC levels and proliferation compared with vehicle. In a mouse model for aggressive neuroblastoma, AURKA inhibitors decreased tumor NMYC levels and tumor volume compared with vehicle. Next steps include identifying additional inhibitors and testing combinations of existing AURKA inhibitors with alternative NMYC-targeted compounds in neuroblastoma models.</p> <p>The Millennium Pharmaceuticals Inc. unit of Takeda Pharmaceutical Co. Ltd. has alisertib, a second-generation AURKA inhibitor, in Phase III testing to treat T cell lymphoma. The compound also is in Phase II and Phase I testing for other cancers. At least six other companies have AURKA inhibitors in Phase II testing or earlier to treat various cancers.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.720 Published online July 25, 2013</p>	Patented by Millennium Pharmaceuticals; licensing status unavailable	<p>Brockmann, M. <i>et al. Cancer Cell</i>; published online June 20, 2013; doi:10.1016/j.ccr.2013.05.005 Contact: Martin Eilers, University of Wuerzburg, Wuerzburg, Germany e-mail: martin.eilers@biozentrum.uni-wuerzburg.de Contact: Louis Chesler, The Institute of Cancer Research, Sutton, U.K. e-mail: louis.chesler@icr.ac.uk</p>
Dermatology				
Wounds; hair loss	Septin 4 (SEPT4; ARTS)	<p><i>In vitro</i> and mouse studies suggest inhibiting SEPT4 could help treat wounds and promote hair follicle regeneration. In <i>Sept4</i> knockout mice, skin tissue showed higher progenitor cell numbers, including hair follicle stem cells, than tissue from wild-type animals. <i>Sept4</i> knockout mice showed faster wound healing and hair follicle regeneration than wild-type controls. Next steps include creating SEPT4 antagonists and determining whether SEPT4 inhibition increases cancer risk.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.721 Published online July 25, 2013</p>	Findings unpatented; mouse models available for licensing	<p>Fuchs, Y. <i>et al. Science</i>; published online June 20, 2013; doi:10.1126/science.1233029 Contact: Hermann Steller, The Rockefeller University, New York, N.Y. e-mail: steller@rockefeller.edu Contact: Elaine Fuchs, same affiliation as above e-mail: fuchslb@rockefeller.edu</p>
Infectious disease				
Bacterial infection	HBV core protein	<p><i>In vitro</i> and mouse studies suggest the arginine-rich domain of HBV core protein could help treat bacterial infections. In various Gram-positive and Gram-negative bacteria including multidrug-resistant strains, the HBV core protein arginine-rich domain had antimicrobial activity. In a mouse model for <i>Staphylococcus aureus</i>-induced sepsis, early intraperitoneal treatment with peptides based on the domain decreased bacterial burden by 100-fold compared with saline treatment and prevented death in all cases. Next steps include testing the peptides in additional infection models.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.722 Published online July 25, 2013</p>	Patent and licensing status unavailable	<p>Chen, H.-L. <i>et al. PLoS Pathog.</i>; published online June 13, 2013; doi:10.1371/journal.ppat.1003425 Contact: Chiahoh Shih, Academia Sinica, Taipei, Taiwan e-mail: cshih@ibms.sinica.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Bacterial infection	Not applicable	<i>In vitro</i> and mouse studies suggest silver could be used to potentiate the effects of antibiotics to treat bacterial infections. Silver has known antimicrobial activity, but its mechanisms of action and synergy with existing antibiotics are not well understood. <i>In vitro</i> , silver increased reactive oxygen species (ROS) production and membrane permeability in bacteria compared with no treatment. In tetracycline-resistant bacteria, silver plus tetracycline at concentrations that were not toxic to human cell lines decreased growth compared with tetracycline alone. In mouse models for peritonitis, urinary tract infection (UTI) or catheter-infected biofilms, silver plus antibiotics, including gentamicin or vancomycin, decreased growth compared with antibiotics alone. Next steps include developing combination formulations of silver and antibiotics. SciBX 6(28); doi:10.1038/scibx.2013.723 Published online July 25, 2013	Patent application filed; licensed to EnBiotix Inc.	Morones-Ramirez, J.R. <i>et al. Sci. Transl. Med.</i> ; published online June 19, 2013; doi:10.1126/scitranslmed.3006276 Contact: James J. Collins, Boston University, Boston, Mass. e-mail: jcollins@bu.edu Contact: Jose Ruben Morones-Ramirez, same affiliation as above e-mail: morones.ruben@gmail.com
Bacterial infection	Unknown	Cell culture studies suggest anthracimycin derivatives could help treat bacterial infections. In bacterial cell culture, anthracimycin inhibited the growth of Gram-positive bacteria, including <i>Bacillus anthracis</i> and methicillin-resistant <i>Staphylococcus aureus</i> (MRSA), but showed little or no activity against Gram-negative bacteria. In culture, a derivative of anthracimycin inhibited the growth of both Gram-positive and Gram-negative bacteria. Next steps include developing more drug-like derivatives of anthracimycin and testing them in mouse models for MRSA infection. SciBX 6(28); doi:10.1038/scibx.2013.724 Published online July 25, 2013	Unpatented; licensing status not applicable	Jang, K.H. <i>et al. Angew. Chem. Int. Ed.</i> ; published online June 17, 2013; doi:10.1002/anie.201302749 Contact: William Fenical, University of California, San Diego, La Jolla, Calif. e-mail: wfenical@ucsd.edu
Ebola	Unknown	Cell culture and mouse studies suggest selective estrogen receptor modulators (SERMs) could help treat Ebola virus infection. In a high throughput, cell-based screen of approved drugs, estrogen receptor antagonists showed the strongest Ebola antiviral activity. In a mouse model for Ebola virus infection, the SERMs clomiphene or toremifene increased survival compared with vehicle. In cells lacking the estrogen receptor, clomiphene and toremifene inhibited Ebola virus infection, suggesting the drugs do not act through the estrogen signaling pathway. Next steps include further evaluation of the compounds in mouse and nonhuman primate infection models. Orion Corp. and Kyowa Hakko Kirin Co. Ltd. market Fareston toremifene citrate to treat breast cancer. Clomiphene is a generic used to treat female infertility. SciBX 6(28); doi:10.1038/scibx.2013.725 Published online July 25, 2013	Patented; available for licensing from Zalicus Inc.	Johansen, L.M. <i>et al. Sci. Transl. Med.</i> ; published online June 19, 2013; doi:10.1126/scitranslmed.3005471 Contact: Lisa M. Johansen, Zalicus Inc., Cambridge, Mass. e-mail: l johansen@zalicus.com Contact: Gene G. Olinger, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Md. e-mail: gene.olinger@us.army.mil

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
HBV	Not applicable	Rodent studies suggest the addition of Baraclade entecavir to plasmid- and adenoviral vector-based vaccination protocols could help treat chronic HBV infection. In woodchucks chronically infected with woodchuck hepatitis virus (WHV), pretreatment with entecavir followed by injection of a DNA vaccine encoding WHV core antigen led to a more potent antiviral immune response than either treatment alone. Next steps could include developing an analogous vaccine candidate for HBV and evaluating it using the same protocol in combination with entecavir. Bristol-Myers Squibb Co. markets Baraclade, a cyclopentile guanosine nucleoside analog, to treat HBV infection. SciBX 6(28); doi:10.1038/scibx.2013.726 Published online July 25, 2013	Patent and licensing status unavailable	Kosinska, A.D. <i>et al. PLoS Pathog.</i> ; published online June 13, 2013; doi:10.1371/journal.ppat.1003391 Contact: Michael Roggendorf, Essen University Hospital, Essen, Germany e-mail: michael.roggendorf@uni-due.de
Tuberculosis	<i>Mycobacterium tuberculosis</i> decaprenylphosphoryl- β -D-ribose 2 \square -oxidase (dprE1); <i>M. tuberculosis</i> molybdopterin biosynthesis protein (moeW)	Mouse and cell culture studies suggest an inhibitor of dprE1 and moeW could help treat drug-resistant tuberculosis infection. In multidrug-resistant and extensively drug-resistant strains of tuberculosis, the small molecule TCA1 showed potent bactericidal activity. In mouse models for acute and chronic <i>M. tuberculosis</i> infection, oral TCA1 decreased infection in the lung and spleen compared with no treatment and without causing weight loss or other adverse effects. In a series of genetic studies, dprE1 and moeW were implicated as the targets of TCA1. Next steps include optimizing the potency and pharmacokinetics of the compound. SciBX 6(28); doi:10.1038/scibx.2013.727 Published online July 25, 2013	Patent application filed; available for licensing	Wang, F. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 17, 2013; doi:10.1073/pnas.1309171110 Contact: Peter G. Schultz, The Scripps Research Institute, La Jolla, Calif. e-mail: schultz@scripps.edu Contact: William R. Jacobs Jr., Albert Einstein College of Medicine of Yeshiva University, Bronx, N.Y. e-mail: jacobsw@hhmi.org
Urinary tract infection (UTI)	Estrogen receptor	Cell culture and mouse studies suggest estradiol could help treat UTIs in postmenopausal women. In two human urothelial cell lines, estradiol increased mRNA expression of five antimicrobial peptides and intracellular uptake of bacteria compared with vehicle. In ovariectomized mice, lower estrogen levels correlated with higher bacterial proliferation in the bladder. Next steps include conducting studies to mitigate estradiol side effects by combining the hormone with other compounds. SciBX 6(28); doi:10.1038/scibx.2013.728 Published online July 25, 2013	Unpatented; licensing status not applicable	Lüthje, P. <i>et al. Sci. Transl. Med.</i> ; published online June 19, 2013; doi:10.1126/scitranslmed.3005574 Contact: Annelie Brauner, Karolinska Institute, Stockholm, Sweden e-mail: annelie.brauner@ki.se
Neurology				
Diabetic neuropathy	Unknown	Cell culture and mouse studies suggest guaifenesin could help treat diabetic neuropathy. In cultured neurons, the (<i>R</i>)-enantiomer of guaifenesin, but not the (<i>S</i>)-enantiomer, increased neurite outgrowth compared with saline control. In a mouse model for streptozotocin-induced diabetes, oral treatment with the (<i>R</i>)-enantiomer of guaifenesin partially prevented the slowing of motor neuron conduction velocity, indicating prevention of peripheral neuropathy. Next steps could include testing the compounds in diabetic patients. Guaifenesin is a generic OTC expectorant. SciBX 6(28); doi:10.1038/scibx.2013.729 Published online July 25, 2013	Patent and licensing status unavailable	Hadimani, M.B. <i>et al. J. Med. Chem.</i> ; published online June 13, 2013; doi:10.1021/jm400401y Contact: Lakshmi P. Kotra, Toronto General Research Institute, University Health Network, Toronto, Ontario, Canada e-mail: lkotra@uhnres.utoronto.ca

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Epilepsy	Neurotrophic tyrosine kinase receptor 2 (NTRK2; TrkB)	<p>Mouse studies suggest NTRK2 inhibitors could help treat temporal lobe epilepsy. Upregulation of NTRK2 signaling is associated with status epilepticus and subsequent temporal lobe epilepsy in patients. In a mouse model for epilepsy, an Ntrk2 inhibitor decreased the frequency and number of seizures, anxiety-like behaviors and damage to hippocampal neurons compared with vehicle. In the treated mice, reductions in frequency and number of seizures persisted for five to six weeks after discontinuation of the inhibitor, whereas vehicle-treated controls showed an increase in seizure frequency and number over the same period. Future studies could include identifying and testing inhibitors of human NTRK2.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.730 Published online July 25, 2013</p>	Patent status undisclosed; unlicensed; available for partnering	<p>Liu, G. <i>et al. Neuron</i>; published online June 20, 2013; doi:10.1016/j.neuron.2013.04.027 Contact: James O. McNamara, Duke University Medical Center, Durham, N.C. e-mail: jmc@neuro.duke.edu</p>
Nerve damage	Purinergic receptor P2Y G protein-coupled 1 (P2RY1; P2Y1)	<p>Cell culture studies identified dual-acting antioxidant and P2Y1 receptor agonist nucleotides that could be useful as neuroprotectants. In a human cell line, the lead nucleotide selectively agonized P2Y1 with an EC₅₀ value of 7 nM. In rat neuronal cell lines, the nucleotide reduced reactive oxygen species (ROS) production with an IC₅₀ value of 80 nM. Next steps could include evaluating the blood brain barrier permeability of the lead nucleotide and its activity on human neurons and glia.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.731 Published online July 25, 2013</p>	Patent application filed; available for licensing	<p>Azran, S. <i>et al. J. Med. Chem.</i>; published online June 10, 2013; doi:10.1021/jm400197m Contact: Bilha Fischer, Bar-Ilan University, Ramat-Gan, Israel e-mail: bilha.fischer@mail.biu.ac.il</p>
Neurology	CREB binding protein (CREBBP; CBP); E1A binding protein p300 (EP300; p300)	<p>Mouse and cell culture studies suggest CBP and p300 agonists could be useful for improving cognitive function. In cell culture, a small molecule activator of the histone/lysine acetyltransferases CBP and p300 formulated in a carbon nanoparticle penetrated into cells and increased histone acetylation compared with vehicle. In mice, intraperitoneal injection of the nanoparticle-formulated CBP and p300 activator increased neurogenesis, duration of memory and histone acetylation levels in the frontal cortex compared with injection of nanoparticle alone. Next steps include testing the compound in mouse models for neurodegenerative disorders including Alzheimer's disease (AD).</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.732 Published online July 25, 2013</p>	Patent pending; licensing status undisclosed	<p>Chatterjee, S. <i>et al. J. Neurosci.</i>; published online June 26, 2013; doi:10.1523/JNEUROSCI.5772-12.2013 Contact: Anne-Laurence Boutillier, University of Strasbourg, Strasbourg, France e-mail: laurette@unistra.fr Contact: Tapas K. Kundu, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, India e-mail: tapas@jncasr.ac.in</p>
Pain	μ-Opioid receptor (OPRM1; MOR); cannabinoid CB1 receptor (CNR1)	<p>SAR and mouse studies identified a bivalent dual MOR agonist and CNR1 antagonist that could be useful for treating pain. In cell culture, compounds containing an α-oxymorphamine MOR agonist pharmacophore linked to a CNR1 antagonist pharmacophore induced clustering and endocytosis of both receptors. In a mouse model for pain, intracerebroventricular and intrathecal injection of the bivalent compound decreased pain compared with injection of a monovalent MOR agonist control and did not lead to tolerance. Next steps could include further chemical optimization.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.733 Published online July 25, 2013</p>	Patent and licensing status undisclosed	<p>Le Naour, M. <i>et al. J. Med. Chem.</i>; published online June 4, 2013; doi:10.1021/jm4005219 Contact: Philip S. Portoghesi, University of Minnesota, Minneapolis, Minn. e-mail: porto001@umn.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Pain	Potassium channel Kv1.2 (KCNA2)	Rat studies suggest targeting <i>KCNA2</i> antisense long noncoding RNA (lncRNA) could help treat neuropathic pain. In a rat model for neuropathic pain, injured dorsal root ganglion neurons showed greater <i>Kcna2</i> antisense lncRNA expression and lower <i>Kcna2</i> protein expression than uninjured nerves. In a rat model for peripheral nerve injury, <i>Kcna2</i> sense RNA normalized <i>Kcna2</i> protein levels and decreased neuropathic pain compared with control RNA. Next steps include testing inhibition of <i>Kcna2</i> lncRNA in animal models for cancer-associated and chronic pain (see Silencing neuropathic pain , page 7).	Unpatented; licensing status not applicable	Zhao, X. <i>et al. Nat. Neurosci.</i> ; published online June 23, 2013; doi:10.1038/nn.3438 Contact: Yuan-Xiang Tao, The Johns Hopkins University School of Medicine, Baltimore, Md. e-mail: ytao1@jhmi.edu
Other				
Poisoning	Solute carrier family 22 organic cation transporter member 2 (SLC22A2; OCT2)	Mouse and cell culture studies suggest inhibiting OCT2 could help decrease oxaliplatin-induced neurotoxicity. In a human cell line, a small molecule OCT2 inhibitor decreased oxaliplatin uptake compared with no treatment. In mice receiving oxaliplatin, <i>Oct2</i> knockout lowered marker expression for oxaliplatin-induced neuropathy compared with no knockout. Next steps include evaluating the ability of OCT2-specific inhibitors in modulating oxaliplatin-induced neuropathy in patients with neurotoxicity. Oxaliplatin is a generic chemotherapeutic.	Unpatented; licensing status not applicable	Sprowl, J.A. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online June 17, 2013; doi:10.1073/pnas.1305321110 Contact: Alex Sparreboom, St. Jude Children's Research Hospital, Memphis, Tenn. e-mail: alex.sparreboom@stjude.org

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
Disease models			
Matrix metalloproteinase 9 (MMP9)-deficient mice for modeling preeclampsia	<p>Mouse fetuses and pregnant mice lacking <i>Mmp9</i> could be useful in identifying therapies to treat preeclampsia. Maternal deficiencies in circulating MMP9 levels are associated with human preeclampsia. In wild-type and <i>Mmp9</i>-deficient pregnant mice, homozygous <i>Mmp9</i> knockout embryos showed defects in implantation and placental development similar to those observed in human preeclampsia. In pregnant mice, homozygous <i>Mmp9</i> knockouts showed maternal symptoms of preeclampsia, including increased blood pressure and proteinuria, whereas heterozygous <i>Mmp9</i> knockouts and wild-type mice did not. Future studies could include utilizing the models to screen potential preeclampsia therapeutics.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.736 Published online July 25, 2013</p>	Patent and licensing status unavailable	<p>Plaks, V. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online June 17, 2013; doi:10.1073/pnas.1309561110 Contact: Zena Werb, University of California, San Francisco, Calif. e-mail: zena.werb@ucsf.edu</p>
Organoid model for colon cancer derived from primary intestinal epithelial cells	<p>Primary intestinal epithelial cells modified to form tumorigenic organoids in 3D cell culture could provide an <i>in vitro</i> model for colon cancer. In 3D cultures of primary intestinal epithelial cells, lentiviral-mediated small hairpin RNA knockdown of <i>adenomatous polyposis coli (APC)</i> led to the production of organoids with genetic features comparable to those in <i>APC</i>-deficient adenomas. In nude mice, injection of <i>APC</i>-deficient organoids led to intestinal cancer-like tumors with cancer stem cell-like properties. Next steps include testing knockdown of additional colon cancer genes in the model.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.737 Published online July 25, 2013</p>	Unpatented; licensing status not applicable	<p>Onuma, K. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online June 17, 2013; doi:10.1073/pnas.1221926110 Contact: Yoshitaka Hippo, National Cancer Center Research Institute, Tokyo, Japan e-mail: yhippo@ncc.go.jp</p>
Drug delivery			
Retinal delivery of gene therapy via intravitreal injection of an engineered adeno-associated viral (AAV) vector	<p>Intravitreal injection of an engineered AAV vector could enable retinal gene therapy. Directed evolution of AAV libraries identified an AAV variant that, after intravitreal injection in normal mice and monkeys, entered photoreceptors and other layers of the outer retina. In mouse models for X-linked retinoschisis or Leber's congenital amaurosis (LCA), intravitreal injection of the AAV variant expressing <i>retinoschisis X-linked juvenile 1 (Rsl; Xlrs1)</i> or <i>retinal pigment epithelium-specific protein 65 kDa (Rpe65)</i> decreased signs of disease and increased retinal function compared with injection of control AAV vectors expressing the respective genes. Future studies could include testing the AAV variant as a gene therapy vector in animal models for other congenital retinal diseases.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.738 Published online July 25, 2013</p>	Patented by The Regents of the University of California; licensed to an undisclosed entity; available for partnering	<p>Dalkara, D. <i>et al. Sci. Transl. Med.</i>; published online June 12, 2013; doi:10.1126/scitranslmed.3005708 Contact: David V. Schaffer, University of California, Berkeley, Calif. e-mail: schaffer@berkeley.edu Contact: John G. Flannery, same affiliation as above e-mail: flannery@berkeley.edu</p>
Serum protease-degradable peptide linkers to control antimicrobial peptide release	<p>Customized peptide linkers that are degraded by serum proteases could be used to control the release of therapeutic peptides from pegylated prodrugs. Antimicrobial peptides were conjugated to polyethylene glycol (PEG) using peptide linkers of up to five amino acids containing sequences recognized by serum proteases. In mouse sera, the release rate of the antimicrobial peptides and resulting antimicrobial activity were modulated in a linker-dependent manner. Ongoing studies include evaluating additional linker peptides to determine the extent that release kinetics can be modulated and applying the approach to other therapeutic peptides.</p> <p>SciBX 6(28); doi:10.1038/scibx.2013.739 Published online July 25, 2013</p>	Patent application filed; available for licensing	<p>Nollmann, F.I. <i>et al. Angew. Chem. Int. Ed.</i>; published online June 13, 2013; doi:10.1002/anie.201301533 Contact: Ralf Hoffmann, Leipzig University, Leipzig, Germany e-mail: hoffmann@chemie.uni-leipzig.de</p>

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Drug platforms			
CpG-depleted adeno-associated virus (AAV) vectors for improved delivery to skeletal muscle	AAV vectors depleted of the toll-like receptor 9 (TLR9) ligand CpG could improve the success of gene therapy for musculoskeletal diseases. In mice injected intramuscularly with an AAV vector expressing a reporter gene, <i>Tlr9</i> -deficient mice had decreased AAV-associated T cell responses, T helper type 1 (Th1) cell responses and major histocompatibility complex class II (MHCII) expression compared with wild-type mice. In the animals, intramuscular injection with an AAV vector that lacked CpG motifs triggered less of an immune response than an AAV-reporter vector that contained CpG motifs. Next steps could include testing the CpG-depleted AAV vectors in dogs and nonhuman primates. SciBX 6(28); doi:10.1038/scibx.2013.740 Published online July 25, 2013	Patent and licensing status unavailable	Faust, S.M. <i>et al. J. Clin. Invest.</i> ; published online June 17, 2013; doi:10.1172/JCI68205 Contact: James M. Wilson, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pa. e-mail: wilsonjm@mail.med.upenn.edu
Crystal structure of variable lymphocyte receptor (VLR) VLRB.aGPA.23 with a tumor-associated glycan antigen	The crystal structure of VLR bound to a tumor-associated glycan antigen could be useful for designing tumor-targeting VLRs for therapeutic and diagnostic applications. VLRs are adaptive immunity proteins from jawless vertebrates that bind glycans with selectivity comparable to that of antibodies. Crystal structure and thermodynamic studies showed that VLRB.aGPA.23 binds the tumor-associated Thomsen-Friedenreich antigen (TF) with four tryptophan residues that create a hydrophobic cage around the target disaccharide. Next steps include developing VLRB.aGPA.23 as a diagnostic reagent and determining its targets for therapeutic applications. SciBX 6(28); doi:10.1038/scibx.2013.741 Published online July 25, 2013	Patents issued and pending covering VLR composition of matter and methods for making and using the VLRs; available for licensing	Luo, M. <i>et al. J. Biol. Chem.</i> ; published online June 19, 2013; doi:10.1074/jbc.M113.480467 Contact: Roy A. Mariuzza, Institute for Bioscience and Biotechnology Research at the University of Maryland, Rockville, Md. e-mail: rmariuzz@umd.edu
Imaging			
Near-infrared fluorescent proteins (iRFPs) that allow multicolor, <i>in vivo</i> imaging	<i>In vitro</i> and mouse studies identified four iRFPs that could be used for noninvasive, multicolor imaging in animals. In HeLa cells and in mice, the new iRFPs produced distinct colors and had sufficient brightness to allow long-term imaging. In mice, two iRFPs could be used simultaneously to image and differentiate between tissues at different depths. Next steps could include testing the agents in additional models and specific processes. SciBX 6(28); doi:10.1038/scibx.2013.742 Published online July 25, 2013	Patent and licensing status unavailable	Shcherbakova, D.M. & Verkhusha, V.V. <i>Nat. Methods</i> ; published online June 16, 2013; doi:10.1038/nmeth.2521 Contact: Vladislav V. Verkhusha, Albert Einstein College of Medicine of Yeshiva University, Bronx, N.Y. e-mail: vladislav.verkhusha@einstein.yu.edu

<i>Rs1</i>	18	Stem cell factor receptor tyrosine kinase	12	Thomsen-Friedenreich antigen	19	Velcade	11
S		Streptozotocin	15	TLR9	19	Vemurafenib	12
SAF-301	2	<i>Sulfatase modifying factor 1</i>	2	TMECG	12	VLR	19
SEPT4	13	<i>SUMF1</i>	2	Toll-like receptor 9	19	VLRB.aGPA.23	19
Septin 4	13	T		Toremifene	14	V-myc myelocytomatosis viral related oncogene neuroblastoma derived	13
SGSH	1	TAU	5	Toremifene citrate	14	X	
Silver	14	TCA1	15	TP73	12	<i>Xlrs1</i>	18
SLC22A2	17	T cell receptor	9	TrkB	16	Z	
SNCA	5	TCR	9	Tumor protein p73	12	Zelboraf	12
SNX-111	8	Tetracycline	14	V		Ziconotide	8
Solute carrier family 22 organic cation transporter member 2	17	TF	19	Vancomycin	14		
				Variable lymphocyte receptor	19		