Jan-Feb-Mar 2015
Volume 16, Issue 1

Targeting Trends

Reporting the latest news in Molecular Surgery

Impairments in gait, posture and complex movement control in rats modeling the multi-system, cholinergic-dopaminergic losses in Parkinson's Disease

by Aaron Kucinski (Collaborators at University Michigan, Ann Arbor: K. Phillips, R. Albin, M. Sarter)

In addition to the primary disease-defining symptoms that result from extensive loss of nigrostriatal dopaminergic neurons, approximately half of patients with Parkinson's Disease (PD) suffer from postural instability, impairments in gait control and a propensity for falls. These symptoms have been associated with losses of cholinergic neurons situated in the basal forebrain (BF) and in the brainstem pedunculopontine nucleus (PPN). We recently developed a test system (Michigan Complex Motor Control Task, MCMCT) for the assessment of fall propensity in rats. Our initial research focusing on the modeling of falls found that cholinergic lesions of the BF in combination with striatal dopamine (DA) lesions (dual lesions, 'DL') generated rats with a high rate of falls that correlated with attentional impairments on an attention task[1]. Given that PPN cholinergic projections have been associated with fall status in PD, we further sought to determine the contribution of PPN cholinergic loss to gait control and falls in rats with cholinergic BF and/or striatal dopamine system losses.

The MCMCT was designed to tax the ability to rapidly correct movement errors when traversing complex rotating surfaces (square rods). Rats were trained to traverse stationary and rotating rods, placed horizontally or at inclines. Traversing rotating rods and avoiding falls required persistent control of gait, limb coordination and carefully timed and placed steps. Following training, rats received cholinergic and/or striatal dopaminergic lesions or sham lesions. Cholinergic lesions were produced by bilateral infusions of 192 IgG-SAP (Cat. #IT-01) or Anti-ChAT-SAP (Cat. #IT-42) into the BF or PPN, respectively. Caudate dopaminergic deafferentation was achieved by bilateral infusions of 6-hydroxydopamine (6-OHDA) into the caudate nucleus. Following surgeries, rats were tested on a 14 day MCMCT test battery with increasingly complex traversal conditions (see Fig. 1).

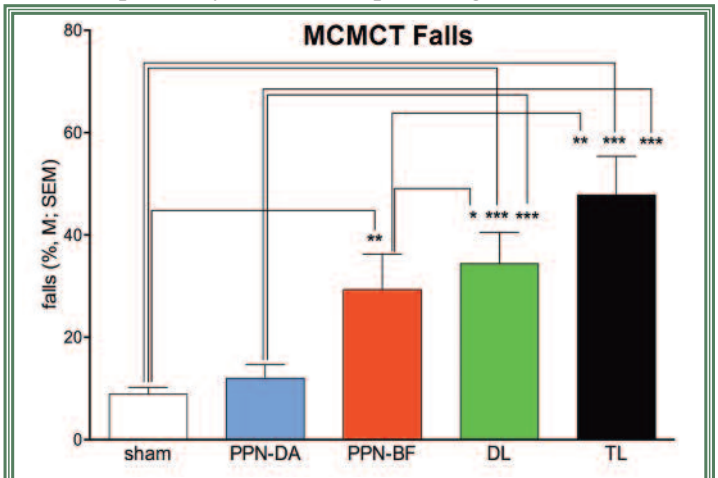

Fig. 1. Rats with PPN + BF cholinergic cell losses, with BF cholinergic and striatal dopamine lesions (DL), and with triple system lesions (TL) fell more than shams from the rotating rod (alternating directions).

The results indicated that rats with losses of PPN and BF cholinergic neurons and striatal dopaminergic inputs fell frequently from the rods (Fig. 1), and that these falls were associated with relatively slow traversal speed and high rate of slips. The performance of rats with losses in all three regions (PPN, BF,

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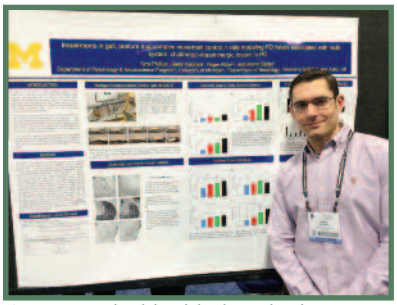
Inside this issue:

Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

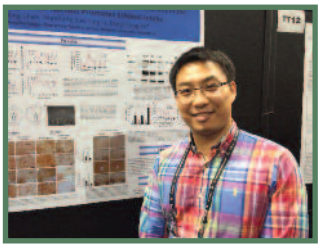
Society for Neuroscience Poster of the Year Award



Aaron Kucinski with the winning poster: Impairments in Gait, Posture and Complex Movement Control in Rats Modeling the Multi-System, Cholinergic-Dopaminergic Losses in PD.

Congratulations to Dr. Kucinski as this year's winner of the SfN Poster of the Year Award for the most interesting work presented using ATS products. You can read a summary of this work from Dr. Sarter's lab in the cover article in this issue of *Targeting Trends*. Here is a small sample of the comments our scientific judges had: "Nice IHC staining with both 192-IgG-SAP (Cat. #IT-01) and anti-ChAT-SAP (Cat. #IT-42)." "It was very cool to see pictures of the actual rat behavior. They showed the Parkinson's rat falling off the run."

Dr. Fu was a strong contender. His lab at Rutgers University used Dermorphin-SAP (Cat. #IT-12) to target mu opioid receptor (MOR) expressing neurons. Their findings indicate that MOR-expressing GABA neurons in the rostromedial tegmental nucleus play a crucial role in the regulation of ethanol consumption, implicating the dysfunction of these neurons likely play a critical role in the pathogenesis of alcoholism, and that these neurons should represent an appropriate target for the development of therapeutic strategies against alcohol use disorders.



Rao Fu with the runner-up poster: Selective Ablation of Mu Opioid Receptor Expressing GABA Neurons in rhe Rostromedial Tegmental Nucleus Promotes Ethanol Intake.

Thank you to the 31 presenters this year. Excellent work!

Upcoming Events

Amer Assoc Immunologists
May 8-12, 2015
New Orleans, LA
Booth #541

Society for Neuroscience
October 17-21, 2015
Chicago, IL
Booth #662

Veterinary Development of Substance P-Saporin (SP-SAP)

Otis, one of the patients with bone cancer who was treated with SP-SAP in the veterinary clinical trial conducted by Dr. Dottie Brown at the University of Pennsylvania.

A groundbreaking pain therapeutic is poised for conditional approval in 2015 to treat bone cancer pain in dogs. The FDA has already approved Minor Use/Minor Species (MUMS) designation for the drug, providing extended market exclusivity to treat the >10,000 annual cases of canine bone cancer-related pain, and the ability to commercialize the drug as soon as conditional approval is given. Given the FDA's receptiveness to the drug, clinical studies are in the planning stages to evaluate its effectiveness in the almost 10 million cases of osteoarthritis in dogs, as well as chronic pain in cats.

The drug, Substance P-Saporin (SP-SAP), has demonstrated remarkable pivotal-study efficacy as viewed in this video of one of the canine patient participants in the pilot veterinary clinical trial (Otis Patient Video, <2 min). Based on the impact of SP-SAP on the observable level of pain in these companion animals, the Center for Veterinary Medicine (CVM) is encouraging a multi-center efficacy trial to gain rapid full-approval for SP-SAP. Contract Research Organizations (CRO's) have been put in place to provide GMP manufacturing, packaging, and labeling of the drug. Four veterinary specialty hospitals across the U.S. have been identified and coordinated for the multi-center efficacy trial. The expected success in this trial will provide full approval for SP-SAP, putting relief from all chronic pain indications within reach for companion dogs. Pain would no longer be a life-threatening disease for family pets.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

The rate of fall of blood glucose determines the necessity of forebrain-projecting catecholaminergic neurons for male rat sympathoadrenal responses.

Jokiaho AJ, Donovan CM, Watts AG.
Diabetes 63(8):2854-2865, 2014.

Different sets of glucosensors detect insulin-induced hypoglycemia depending on the onset rate. This detection controls the activation of sympathoadrenal counterregulatory responses (CRRs). Slow onset hypoglycemia, common with insulin therapy, is detected by glucosensors in the portal-mesenteric veins. Fast onset is detected by brain elements. The authors lesioned hindbrain catecholaminergic neurons to determine which set of responses-they interact with. Rats received 42 ng bilateral injections of Anti-DBH-SAP (Cat. #IT-03) into the paraventricular nucleus of the hypothalamus. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The data indicate that these neurons are critical for detection of slow-onset insulin-induced hypoglycemia.

Intratumoral anti-HuD immunotoxin therapy for small cell lung cancer and neuroblastoma.

Ehrlich D, Wang B, Lu W, Dowling P, Yuan R.
J Hematol Oncol 7(1):91, 2014.

HuD protein is a 40-kDa neuronal RNA-binding protein that is expressed in 100% of small cell lung cancer (SCLC) tumor cells. An anti-HuD monoclonal was biotinylated and combined with Streptavidin-ZAP (Cat. #IT-27); this conjugate was tested both *in vitro* and *in vivo*. Anti-HuD-SAP eliminated NCI-H69 and Neuro-2a cells at an EC_{50} of <0.5 µg/ml. 1 mg/kg of the conjugate injected directly into subcutaneous tumors generated in mice resulted in a temporary lack of tumor growth or regression of the tumor.

Respiratory function after selective respiratory motor neuron death from intrapleural CTB-saporin injections.

Nichols NL, Vinit S, Bauernschmidt L, Mitchell GS.
Exp Neurol Epub2014.

Amyotrophic lateral sclerosis (ALS) ultimately causes death from ventilator failure. Genetic models of ALS suffer from high variability of the rate, timing, and extent of respiratory motor neuron death. The authors created a novel model of induced respiratory motor neuron death using CTB-SAP (Cat. #IT-14). Rats received 25 µg or 50 µg intrapleural injections of CTB-SAP; Saporin (Cat. #PR-01) was used as a control. After 7 days, motor neuron survival approximated what is seen in end-stage ALS rats, while there was minimal cell death in other brainstem or spinal cord regions. CTB-SAP also caused microglial activation, decreased breathing during chemoreceptor stimulation, and diminished phrenic motor output in anesthetized rats – all hallmarks of ALS.

Hypocretin/orexin antagonism enhances sleep-related adenosine and GABA neurotransmission in rat basal forebrain.

Vazquez-DeRose J, Schwartz MD, Nguyen AT, Warrier DR, Gulati S, Mathew TK, Neylan TC, Kilduff TS.
Brain Struct Funct Epub2014.

The basal forebrain (BF) is one of the regions receiving excitatory input from orexin neurons. The authors investigated the hypothesis that orexin antagonists induce sleep at least in part by interfering with the facilitation of BF neurons. Rats received bilateral 500-ng injections of 192-IgG-SAP (Cat. #IT-01) into the BF. Lesioned animals displayed no abnormal responses to a benzodiazepine agonist or vehicle. An orexin antagonist, however, was less effective than the control at inducing sleep in lesioned rats.

Increasing inflationary T-cell responses following transient depletion of MCMV-specific memory T cells.

Sims S, Klenerman P.
Eur J Immunol Epub2014.

The standard CD8+ T-cell response to infection is a rapid proliferation followed by a reduction in number after the infection is cleared. Murine cytomegalovirus is an exception in that an infection generates a life-long latency with low-level sporadic replication. Immunodominant cells accumulate over time and stabilize at a high frequency. The authors examined a paradoxical boost following depletion of these cells with an M38 antibody attached to Streptavidin-ZAP (Cat. #IT-27). Mice were treated with 44 pM intraperitoneal injections. M38 is an epitope present on the effector CD8+ T cells. Following a significant depletion of cells, the population rebounded and reached a higher percentage of total CD8+ T-cells than before the depletion.

A combination of targeted toxin technology and the piggyBac-mediated gene transfer system enables efficient isolation of stable transfectants in nonhuman mammalian cells.

Sato M, Inada E, Saitoh I, Matsumoto Y, Ohtsuka M, Miura H, Nakamura S, Sakurai T, Watanabe S.
Biotechnol J Epub2014.

In this work the authors developed a new transfection strategy that takes advantage of the fact that many cell lines endogenously express α-1,3-galactosyltransferase (α-Gal), the target of rIB4-SAP (Cat. #IT-10). After transfection low expressing or non-transfected cells are killed by an application of rIB4-SAP at 80 µg/ml for 2 hours. The surviving cells eventually express α-Gal again, and require no selective agent to maintain expression of the gene of interest. These transfected cells can be transfected again using the same method.

Cholinergic neurons of the basal forebrain mediate biochemical and electrophysiological mechanisms underlying sleep homeostasis.

Kalinchuk AV, Porkka-Heiskanen T, McCarley RW, Basheer R.
Eur J Neurosci Epub2014.

Previous work has indicated that non-rapid eye movement during recovery sleep after sleep deprivation requires cholinergic neurons in the BF. The authors examined how BF cholinergic neurons affect the levels of HSP markers during sleep deprivation. Rats received 230-ng injections of 192-IgG-SAP (Cat. #IT-01) into the horizontal limb of the diagonal band/substantia innominata/

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

magnocellular preoptic area. The results indicate that cholinergic neurons in the BF are important for regulating the biochemical and EEG mechanisms that contribute to HSP.

Eye-specific retinogeniculate segregation proceeds normally following disruption of patterned spontaneous retinal activity.

Speer CM, Sun C, Liets LC, Stafford BK, Chapman B, Cheng HJ.
Neural Dev 9(1):25, 2014.

The authors administered 0.88-1.66 µg of an Anti-VaChT-SAP custom conjugate to ferrets with an intraocular injection. Although the lesioned animals demonstrated normal eye-specific retinogeniculate development, there were significant abnormalities in spontaneous retinal activity. These differences in activity manifested themselves as eye-specific segregation defects.

Role of spinal bombesin-responsive neurons in nonhistaminergic itch.

Akiyama T, Tominaga M, Takamori K, Carstens MI, Carstens E.
J Neurophysiol 112(9):2283-2289, 2014.

Recent papers have demonstrated that pruritogen-evoked scratching behavior is reduced or eliminated by intrathecal injection of Bombesin-SAP (Cat. #IT-40). In this work the authors build on those data by investigating if spinal neurons that are responsive to pruritogens administered intradermally are also responsive to a spinal infusion of bombesin. Through the use of intradermal chloroquine injections, spinal superfusion of bombesin, and noxious pinch, the overlap of neurons processing itch and nociception was examined. The results demonstrate that chloroquine- and bombesin-sensitive neurons are involved in the transmission of itch, and that these are a separate neuronal population from those involved in nociception.

Don't see your publication here? Send us a PDF at ats@ATSbio.com and we'll be sure to review it in our next issue.

Efficient elimination of CD103-expressing cells by anti-CD103 antibody drug conjugates in immunocompetent mice.

Mang Y, Zhao Z, Zeng Z, Wu X, Li Z, Zhang L.
Int Immunopharmacol 24(1):119-127, 2015.

Previous work has demonstrated that a custom M290-SAP conjugate promoted the long-term survival of pancreatic islet allografts by reducing the number of CD103+ cells. M290 is an antibody that targets CD103. Systemic use of the saporin conjugate can result in toxicity and bystander effects to the animal. In this work the authors used M290 conjugated to three different cytotoxic agents in order to avoid these bystander effects. The various reagents were compared in several assays, including internalization studies, flow cytometry, and cytotoxicity studies. The results indicate that the alternative cytotoxic drugs can be used systemically with M290 to eliminate CD103+ cells.

Improvements in memory after medial septum stimulation are associated with changes in hippocampal cholinergic activity and neurogenesis.

Jeong da U, Lee JE, Lee SE, Chang WS, Kim SJ, Chang JW.
Biomed Res Int 2014:568587, 2014.

Deep brain stimulation (DBS) is a technique by which electrical impulses are applied to specific areas of the brain as therapy for various disorders. In this work the authors examined the mechanisms by which DBS can treat dementia. Rats received 5.04 µg intracerebroventricular injections of 192-IgG-SAP (Cat. #IT-01); some rats also received an electrode implanted into the medial septum. Lesioned animals displayed deficits in water maze testing – this deficit was eliminated for the group that received electrical stimulation to the medial septum. The stimulated group also displayed an increase in hippocampal cholinergic activity as well as neurogenesis, indicating that DBS has therapeutic potential.

NK1-receptor-expressing paraventricular nucleus neurones modulate daily variation in heart rate and stress-induced changes in heart rate variability.

Feetham CH, Barrett-Jolley R.
Physiol Rep 2(12):e12207, 2014.

Neurons in the paraventricular nucleus (PVN) project to the medulla and spinal cord, regulating heart rate and blood pressure. Although the activity of these neurons becomes elevated during heart failure, their role in overall cardiovascular control is unclear. The authors lesioned the PVN of rats with 2 ng injections of SSP-SAP (Cat. #IT-11). Heart rate variability during the experiment was measured using a high/low frequency ratio in response to psychological stress. The variability response of lesioned rats was lower than that of controls, and a shift in daily heart rate variation was seen as well. The authors conclude that neurokinin-1 expressing neurons in the PVN couple the cardiovascular system to the daily heart rate as well as the sympathetic response to psychological stress.

Targeted Toxin-Based Selectable Drug-Free Enrichment of Mammalian Cells with High Transgene Expression.

Sato M, Akasaka E, Saitoh I, Ohtsuka M, Nakamura S, Sakurai T, Watanabe S.
Biology 2(1):341-355, 2013.

Cell transfection is a powerful tool for evaluation of function and expression of newly discovered genes as well as for both small and large scale eukaryotic expression of proteins. Most transfection strategies require a selection agent to eliminate cells that do not internalize the plasmid containing the gene of interest. Subsequent maintenance of the tranfected cells requires the presence of the selection agent, and the expression levels of the gene of interest have to be evaluated on a cell by cell basis. In this work the authors designed a system utilizing 50 µg/ml rIB4-SAP (Cat. #IT-10) to eliminate non-transfected cells and select for strong expression of the gene of interest. The data demonstrate that this technique will generate stable transfected cells that express the gene of interest at high levels.

Targeting Talk: Product Q&A

Q: Our lab is getting ready to begin a project using one of your targeted toxins. We already did a preliminary experiment to try out the material, but we have a couple of questions before we start the larger project. First, do you have any protocols or references for injecting intrathecally?

A: Thank you for your inquiry. We appreciate the opportunity to get involved in projects before they begin. At Advanced Targeting Systems, we do not do any *in vivo* work, just *in vitro*, however we have collaborated with many fine laboratories that have good experience with intrathecal injections. If you search PubMed with the keywords 'saporin' and 'intrathecal' you will be able to view 36 references that will give you good information on techniques and protocols.

Prior to beginning your project you will want to submit your animal care guidelines to your IACUC committee. Turner *et al.* published an article that will be helpful regarding intrathecal injections.[1]

Q: The second question is in two parts: 1) how do we determine the appropriate dose, and 2) how do we know saporin is not killing indiscriminately at that dose?

A: You should always use a control when determining the appropriate dose. A basic premise of the ATS targeting technology is that if a control (saporin alone or a control conjugate) evokes a response, then the dose is too high. Whenever a new shipment of targeted toxin is received, the proper working dilution should be ascertained before beginning a project. The targeted toxin data sheet states:
"There may be lot-to-lot variation in material; working dilutions must be determined by end user. If this is a new lot, assess the proper working dilution before beginning a full experimental protocol."

If you search on the ATS website for the species and route of administration you plan to use, you can look through the quarterly summaries of publications and see the dose that was used for that particular study. That will give you a ballpark range in which to start your dose titration. Just keep in mind: if the control kills cells, the dose is too high.

1. Turner *et al.*, Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider, *J Am Assoc Lab Anim Sci,* 50(5): 600–613, 2011.

Q&A Products

Control Conjugates

Blank-CTA
for peptide-targeted CTA conjugates (IT-61)

Blank-SAP
for peptide-targeted SAP conjugates (IT-21)

Fab IgG-SAP
for goat IgG Fab-ZAP secondary conjugates (IT-67)

Goat IgG-SAP
for goat IgG-containing immunolesioning agents (IT-19)

Human IgG-SAP
for human IgG-containing immunolesioning agents (IT-49)

Mouse IgG-SAP
for mouse IgG-containing immunolesioning agents (IT-18)

Mouse IgM-SAP
for mouse IgM-containing immunolesioning agents (IT-41)

Rabbit IgG-SAP
for rabbit IgG-containing immunolesioning agents (IT-35)

Rat IgG-SAP
for rat IgG-containing immunolesioning agents (IT-17)

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Modeling Fall Propensity in Parkinson's Disease

(continued from page 1)

and DA; triple lesions, 'TL') was not more severely impaired than following combined BF cholinergic and striatal DA lesions (DL), however, some abnormal gait characteristics were observed such as ballistic recovery movements and slip-triggered switches to symmetrical locomotion ('galloping'). Furthermore, rats with only cholinergic cell losses (PPN and BF) fell more than shams on more complex rod traversal conditions (rod rotating in alternating directions) (Fig.1). Histological analysis showed that infusions of 192-IgG saporin into the BF removed cholinergic neurons primarily from the nucleus basalis of Meynert and the more ventral substantia innominata (Fig. 2 a,b) and anti-ChAT-SAP infusions into the PPN resulted in the almost complete loss of cholinergic neurons in this region (Fig. 2 c,d). In total, these results support a role of cholinergic systems in falls and gait control in PD and further support the hypothesis that BF cholinergic-striatal disruption of attentional-motor interactions, proposed to reflect impaired attentional control of posture, gait and movement, is a primary source of falls.

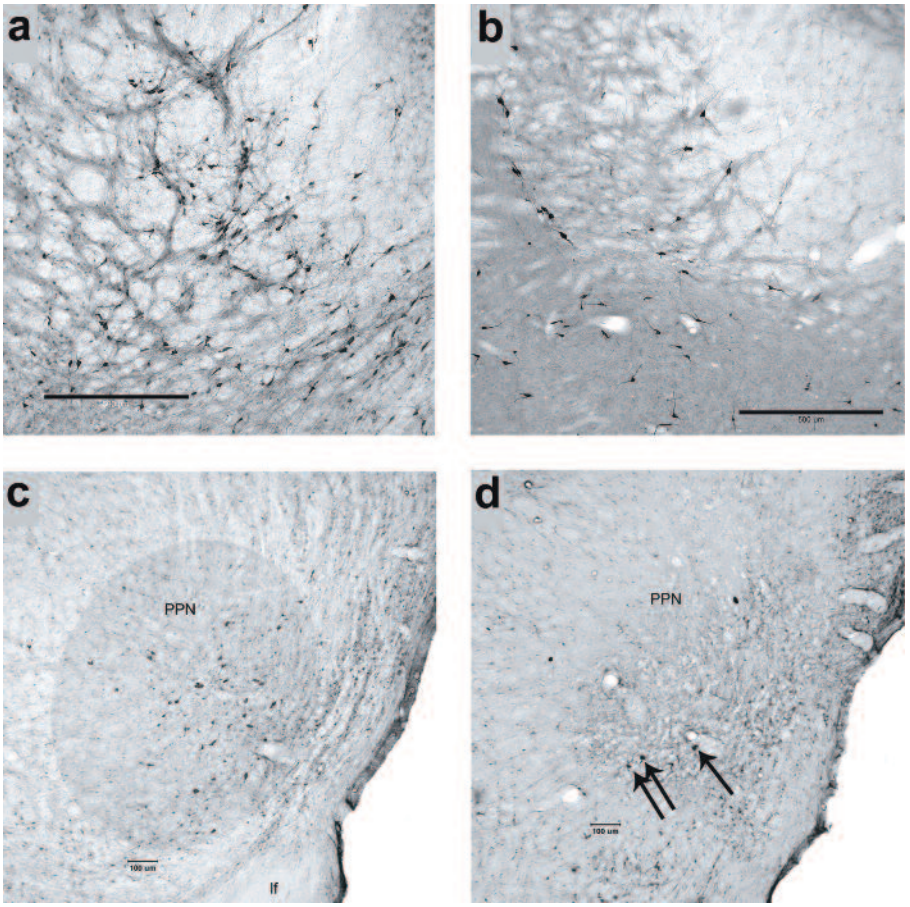

Fig. 2. (a) ChAT immunoreactivity on a coronal section of the BF from a sham-lesioned rat. (b) Infusions of 192-IgG saporin removed 70-90% of the cholinergic neurons in this region. (c) Cholinergic neurons in the intact PPN (shaded region; ChAT-immunoreactivity). d) Infusions of anti-ChAT-SAP lesioned >90% of the neurons (arrows point to neurons spared by the toxin). Scales in b and c are 500µm and in d and e are 100µm.

References

1. Kucinski A, Paolone G, Bradshaw M, Albin RL, Sarter M. Modeling fall propensity in Parkinson's disease: Deficits in the attentional control of complex movements in rats with cortical-cholinergic and striatal-dopaminergic deafferentation. *J Neurosci* 33:16522-39, 2013.

Targeting Teaser Solution

The solution to the puzzle was:

Jumbles: LABRADOR
VETERINARY
ROTTWEILER
TRANSLATION
SIGNIFCANT

Why the zombie wanted to be a neuroscientist.

Answer: He loved... BRAINS!

Solve this quarter's teaser at www.ATSbio.com/news/15q1_teaser.html

Congratulations to the puzzle solvers from last quarter. Each winner will receive an ATS 2015 calendar.

LAST QUARTER'S WINNERS: Jheem D. Medh, California State Univ Northridge * Glenn H. Kageyama, Cal Poly Pomona Univ * Dave Ginsbert, Molecular Innovations * Judene Bliss, Roswell Park Cancer Institute * Daniel Pekala, Charles River Laboratories * Joan Schein, Biochain * Seto Chice, SUNY HSC at Brooklyn * Bill Henry, Rhode Island Hospital

Targeting Tools: New ZAP Kit and Anti-CD44-SAP

ZAP SrB Development Kit

The ZAP Sulforhodamine B (SrB; Cat. #KIT-SrB-Z) Development kit contains all of the materials needed to introduce a quantitative staining assay to your lab. Preferred by the National Cancer Institute for high-throughput drug screening, SrB quantitatively stains cellular proteins in an accurate and reproducible manner. Refined and honed over years of use in testing Saporin-conjugate products, the ZAP SrB kit makes development of cytotoxicity assays efficient, time-flexible, and incredibly consistent. Sulforhodamine B is easily detectible with standard optical plate readers capable of readout between 550-580 nm. Each ZAP SrB kit comes with enough reagents for 1000 tests.

The ZAP Sulforhodamine B (SRB) assay is used for cell density determination, much like MTS, MTT, or XTT. However, rather than measuring cell metabolism, the SrB assay is based on the measurement of cellular protein content. The ZAP SrB Kit and protocol are optimized for the toxicity screening of compounds to adherent cells in a 96-well format. After incubation, cell monolayers are fixed and stained, after which the excess dye is removed. The protein-bound dye is solubilized for OD determination at 564 nm using a microplate reader. The SRB assay provides a colorimetric end point that is visible to the naked eye. In addition, SrB is indefinitely stable; meaning the stain can be applied to the protein, washed and dried, and then left for weeks before resolubilizing and reading in a plate reader. The end point is also non-destructive because the stain is all that is resolubilized, the protein remains fixed to the plate so that the procedure may be repeated again and again. The ZAP SrB Development Kit provides a sensitive measure of drug-induced cytotoxicity, is useful in quantitating clonogenicity, proliferation, and is well suited to high-volume, automated drug screening.

Anti-CD44-SAP

This targeted toxin is a conjugate of a mouse-specific CD44 antibody (clone IM7) and the ribosome-inactivating protein, Saporin. Anti-CD44-SAP (Cat. #IT-72) eliminates murine cells that express all isoforms of the CD44 receptor.

CD44 is a receptor for hyaluronic acid and also interacts with other ligands, such as osteopontin, collagens, and matrix metalloproteinases. CD44 participates in a wide variety of cellular functions such as lymphocyte activation, recirculation and homing, hematopoieses, and tumor metastasis. CD44 has been considered an activity marker and potential novel therapeutic target in multiple sclerosis and is associated with relapses in non-small cell lung cancers.

Beta-Testing Program

Nociceptin-SAP

Eliminates nociceptin-receptor expressing cells.

Octreotide-SAP

Eliminates cells that express somatostatin receptors.

Azido-ZAP

Combines with an alkyne-containing molecule in a click chemistry reaction to eliminate molecules containing a free alkyne group.

Beta Products have not been characterized or reported in scientific literature. This provides researchers with special Beta-pricing and the opportunity to be the first to publish using the material. The researcher who first publishes data will receive a $500 credit for use on ATS products.

Kermit practices CPR on his tiger friend.

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§ or anything recognized on the cell surface and internalized.

The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.

Cells that do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.

SAPORIN inactivates the ribosomes.

The result is .

Targeting Teaser

Unscramble these five Jumbles **taken from the cover story**, one letter to each block, to solve the puzzle.

INSTOPPERY

EXCLOMP

STARVERE

COLOTOMONI

REIGNCOLICH

How using saporin conjugates affects the scientist's New Year.

Arrange the circled letters to form the answer, as suggested by the above clue.

ANSWER:
IT KEEPS RESEARCH RIGHT ON . . . !

WIN!

SOLVE the puzzle online with the correct solution by March 31, 2015.

WIN a 2015 ATS Calendar!

www.atsbio.com/news/15q1_teaser.html

Apr-May-Jun 2015
Volume 16, Issue 2

Targeting Trends

Reporting the latest news in Molecular Surgery

Drug-free selection of stable transfectants using targeted toxin technology and a vector expressing cell-surface carbohydrate-digesting enzyme

by Masahiro Sato[1] (Ph. D.) and Satoshi Watanabe[2] (Ph. D.)

[1]Section of Gene Expression Regulation, Frontier Science Research Center, Kagoshima University, Kagoshima 890-8544, Japan: [2]Animal Genome Research Unit, Division of Animal Science, National Institute of Agrobiological Sciences, Ibaraki 305-8602, Japan

Isolation of stable transfectants is one of the important steps for exploring biological functions of gene of interest. Most studies have employed drug resistance genes, such as the neomycin resistance gene (*neo*), to eliminate unwanted, untransfected cells after transfection. In such cases, the drug resistance genes are integrated into host chromosomes upon transfection so that they synthesize proteins capable of degrading drugs present in medium. However, this method often causes unwanted byproducts that are occasionally toxic to cells if they are continuously cultivated in the drug-containing medium. Therefore, drug-free selection of stable transfectants is necessary. Previously, we have assessed this problem and have provided a way to obtain transfectants efficiently in the absence of drug selection. Our system is based on co-transfection with a vector carrying a gene of interest (GOI) and a vector (pCAG/EndoGalC) carrying a gene encoding *Clostridium perfringens*-derived endo-ß-galactosidase C (EndoGalC), which cleaves a specific cell-surface carbohydrate, called the α-Gal epitope, expressed in most *(continued on page 6)*

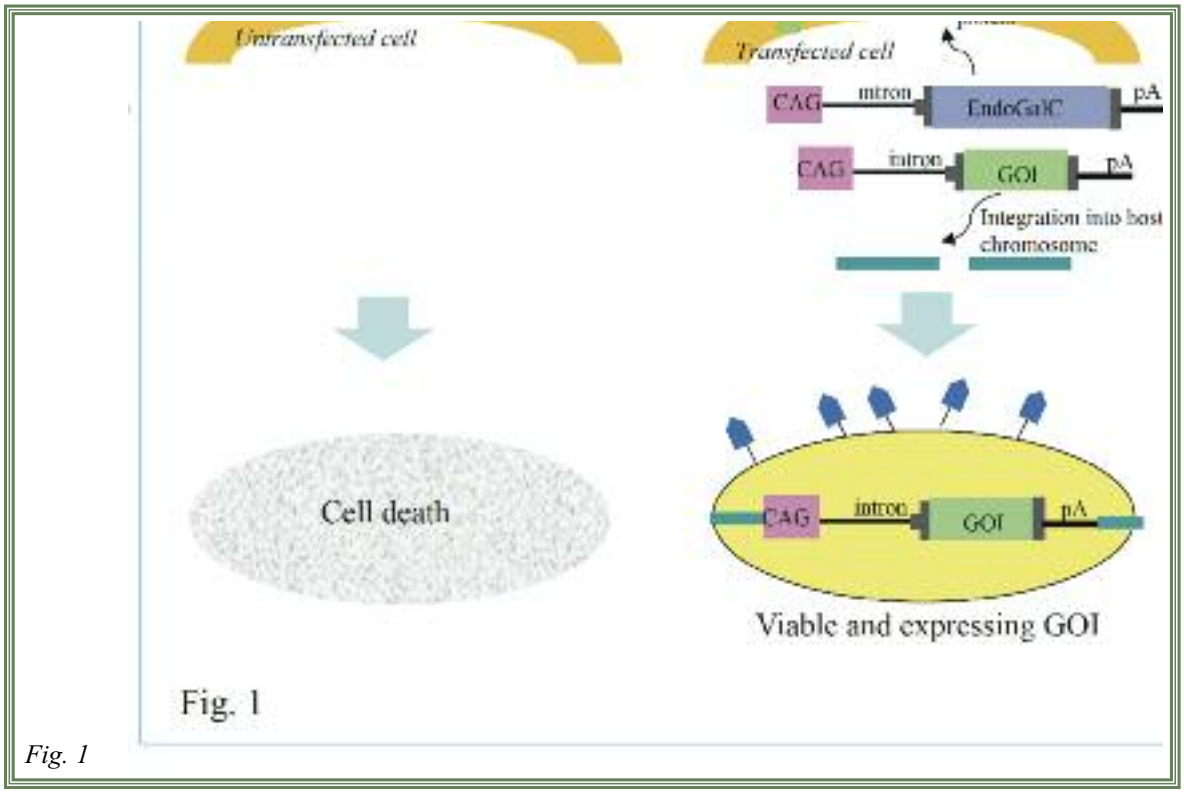

Fig. 1

Inside this issue:

Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Beta-Testing Program: Great Price / Great Opportunity

ATS is pleased to announce Beta-release of a wide array of targeted toxins for use in eliminating specific cell types. This Beta-Testing Program will make new conjugates available to our customers sooner.

Each of the Beta products has:

Saporin activity confirmed,
Peptide sequences published/confirmed, and/or
Antibody binding specificity published/confirmed.

Check out these Beta Products - Available Now!

Nociceptin-SAP

Eliminates nociceptin-receptor expressing cells.

This targeted toxin recognizes cells that express the nociceptin receptor. Nociceptin-SAP is a bonded toxin between nociceptin and the secondary conjugate Streptavidin-ZAP (IT-27) containing the ribosome-inactivating protein, saporin. Nociceptin (Orphanin FQ) is a 17-amino acid peptide widely distributed within the central and peripheral nervous system functioning as an endogenous agonist of the Nociceptin receptor (NOP) formerly known as the opioid receptor-like 1 receptor (ORL1). Nociceptin has been confirmed to play a role in a variety of physiological functions involving not only the CNS and PNS but non-neuronal systems as well. These functions include pain, gastrointestinal motility, locomotion, learning and memory, neurotransmitter and hormone release, renal function, neuronal differentiation, sexual and reproductive behavior and anxiety.

Octreotide-SAP

Eliminates cells that express somatostatin receptors.

Octreotide-SAP is a bonded toxin between octreotide peptide and the ribosome-inactivating protein, saporin. Octreotide is a somatostatin analog and binds to somatostatin receptors on cell surfaces, predominantly somatostatin receptor subtypes 2 & 5. It is an octapeptide that mimics natural somatostatin pharmacologically, though it is a more potent inhibitor of growth hormone, glucagon, and insulin secretion than the natural hormone and has a much longer half-life. Octreotide affects neurotransmission and cell proliferation via interaction with G protein-coupled somatostatin receptors and inhibition of the release of numerous secondary hormones. It is indicated for symptomatic treatment of carcinoid syndrome and acromegaly. It is also finding increased use in treatment of polycystic diseases of the liver and kidney. Octreotide-SAP eliminates cells that express somatostatin receptors.

Azido-ZAP

Combines with alkyne-containing molecule in click chemistry reaction to eliminate molecules containing a free alkyne group.

Click chemistry can be used when methods such as direct labeling or the use of antibodies are not applicable nor efficient. The click chemistry label is small enough that tagged molecules (e.g., nucleotides, sugars, and amino acids) are acceptable substrates for the enzymes that assemble these building blocks into biopolymers. The small size of click detection molecules allows them to easily penetrate complex samples, including intact, supercoiled DNA, with only mild permeabilization required.

Beta Products have not been characterized or reported in scientific literature. This provides researchers with special Beta-pricing and the opportunity to be the first to publish using the material. The researcher who first publishes data will receive a $500 credit for use on ATS products.

Targeting Teaser

Last quarter's puzzle was posted incorrectly online. We are sorry for any inconvenience. It has been corrected and can be solved online. Win a jigsaw puzzle!

Solve the Teaser at www.ATSbio.com/news/15q2_teaser.html

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Cortically projecting basal forebrain parvalbumin neurons regulate cortical gamma band oscillations.

Kim T, Thankachan S, McKenna JT, McNally JM, Yang C, Choi JH, Chen L, Kocsis B, Deisseroth K, Strecker RE, Basheer R, Brown RE, McCarley RW.

Proc Natl Acad Sci U S A Epub2015.

Measurements of cortical EEG capture gamma band oscillations (GBO). Abnormalities in these GBO have been found in some neuropsychiatric disorders such as Alzheimer's disease and schizophrenia. The authors analyzed GBO neuronal groups by administering 650-ng bilateral icv injections of mu p75-SAP (Cat. #IT-16) to mice to determine the role of basal forebrain cholinergic neurons in the generation of GBO. The results indicate GABAergic basal forebrain neurons containing parvalbumin were important for GBO integrity, but cholinergic neurons in the basal forebrain were not involved.

alphaCGRP is essential for algesic exocytotic mobilization of TRPV1 channels in peptidergic nociceptors.

Devesa I, Ferrandiz-Huertas C, Mathivanan S, Wolf C, Lujan R, Changeux JP, Ferrer-Montiel A.

Proc Natl Acad Sci U S A 111(51):18345-18350, 2014.

The sensitization of transient receptor potential vanilloid 1 (TRPV1) can lead to the development and maintenance of chronic pathological pain conditions. In this work the authors determined that TRPV1 receptors use membrane insertion mechanisms in order to potentiate neuronal excitability. In order to specifically link this activity to peptidergic neurons the authors treated rat primary dorsal root ganglion cultures with 10 mM rIB4-SAP (Cat. #IT-10) to deplete the non-peptidergic neurons.

Monoclonal Antibodies Targeting LecLex-Related Glycans with Potent Anti-Tumor Activity.

Jia CX, Vankemmelbeke M, McIntosh RS, Clarke PA, Moss R, Parsons T, Spendlove I, Zaitoun AM, Madhusudan S, Durrant LG.

Clin Cancer Res 2015.

In this work the authors characterized two monoclonal antibodies that target glycans containing Lewis carbohydrate antigens. One of the methods used was to combine varying concentrations of the antibodies with 50 ng mouse Fab-ZAP (Cat. #IT-48) and apply the conjugates to cells for 72 hours. The antibodies were demonstrated to have efficient internalization, supported by potent *in vivo* anti-tumor activity.

Light-controlled endosomal escape of the novel CD133-targeting immunotoxin AC133-saporin by photochemical internalization - A minimally invasive cancer stem cell-targeting strategy.

Bostad M, Olsen CE, Peng Q, Berg K, Hogset A, Selbo PK.

J Control Release 206(28):37–48, 2015.

Previously the authors demonstrated the use of photochemical internalization of a custom conjugate consisting of a CD133 antibody coupled to saporin (ATS Custom conjugation). Several cancer cell lines were plated, and incubated in the presence of a photosensitizer with either CD133-SAP at 8.6 pM or Saporin (Cat. #PR-01) at 24 pM. The different concentrations equalized the number of saporin molecules in each sample. A light source was used to initiate the internalization of the molecules. The results indicate that this is a viable strategy for the targeted treatment of cancer stem cells.

High-content analysis of antibody phage-display library selection outputs identifies tumor selective macropinocytosis-dependent rapidly internalizing antibodies.

Ha KD, Bidlingmaier SM, Zhang Y, Su Y, Liu B.

Mol Cell Proteomics 13(12):3320-3331, 2014.

Macropinocytosis, the internalization of large endocytic vesicles called macropinosomes, is upregulated in Ras-transformed cancers. To date, large-scale antibody generation strategies have not incorporated a selection method for antibodies. In this work the authors demonstrate screening and validation of the antibodies that utilize the macropinosome pathway. One method used was to biotinylate the antibodies and combine them with Streptavidin-ZAP (Cat. #IT-27) at a 1:1 ratio. The conjugate was applied to cells in a concentration curve starting at 200 nM in order to demonstrate internalization and cell killing.

T-box transcription regulator Tbr2 is essential for the formation and maintenance of Opn4/melanopsin-expressing intrinsically photosensitive retinal ganglion cells.

Mao CA, Li H, Zhang Z, Kiyama T, Panda S, Hattar S, Ribelayga CP, Mills SL, Wang SW.

J Neurosci 34(39):13083-13095, 2014.

Opsin 4/melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) are responsible for controlling non-image-forming visual functions in the retina. The findings show that opsin 4 is only expressed in Tbr2-positive ipRGCs, no ipRGCs are found if Tbr2 is deleted before RGC specialization, and most ipRGCs are eliminated when Tbr2 is deleted from established ipRGCs. An antibody against melanopsin (Cat. #AB-N39) was used at a 1:1000 dilution for immunohistochemical analyses.

TrkA in vivo function is negatively regulated by ubiquitination.

Kiris E, Wang T, Yanpallewar S, Dorsey SG, Becker J, Bavari S, Palko ME, Coppola V, Tessarollo L.

J Neurosci 34(11):4090-4098, 2014.

The high affinity nerve growth factor receptor, trkA, plays an intrinsic role in the regulation of various aspects of the mammalian nervous system. The post-translational attachment of ubiquitin to trkA plays a role in the final disposition and function of many proteins; in this work the authors investigate the result of trkA ubiquitination. By removing a 3 amino acid sequence from the receptor the ubiquitination of TrkA was reduced which resulted in an increase in TrkA protein levels and activity. In mice containing this mutation, the rise in TrkA activity was accompanied by enhanced thermal sensitivity and inflammatory pain. Anti-trkA (Cat. #AB-N03) was used at a concentration of 1:500 in immunohistochemistry.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Characteristic patterns of dendritic remodeling in early-stage glaucoma: evidence from genetically identified retinal ganglion cell types.

El-Danaf RN, Huberman AD.

J Neurosci 35(6):2329-2343, 2015.

The loss of retinal ganglion cells (RGC) is the second-most common cause of blindness worldwide. Using several mouse transgenic cell lines, the authors investigated the changes that occur on the establishment of elevated ocular pressure. Anti-melanopsin (Cat. #AB-N39) at 1:1000 was used to illuminate the morphology of the M1 intrinsically photosensitive RGC.

Individual Differences in Acute Pain-induced Endogenous Analgesia Predict Time to Resolution of Postoperative Pain in the Rat.

Peters CM, Hayashida KI, Suto T, Houle TT, Aschenbrenner CA, Martin TJ, Eisenach JC.

Anesthesiology 2015.

The authors investigated the relationship between preoperative Conditioned Pain Modulation (CPM) and the time course of recovery from surgery. CPM was evaluated using forepaw capsaicin injections into rats. During the study, lesioned rats received 5-µg intrathecal injections of anti-DBH-SAP (Cat. #IT-03), followed 14 days later by a partial L5 spinal nerve ligation surgery. Mouse-IgG-SAP (Cat. #IT-18) was used as a control. CPM was partially blocked in the lesioned animals, suggesting descending noradrenergic signaling is important in the time course of recovery from surgery.

New mouse retinal stroke model reveals direction-selective circuit damage linked to permanent optokinetic response loss.

Joly S, Guzik-Kornacka A, Schwab ME, Pernet V.

Invest Ophthalmol Vis Sci 55(7):4476-4489, 2014.

The authors used a mouse model of 'retinal stroke' to better delineate the optokinetic response deficits at the cellular level. Damage was found in the processes of starburst amacrine cells (SACs), and to a lesser extent, the dendrites. Anti-melanopsin (Cat. #AB-N38) at 1:2500 was used for immunohistochemistry.

Neutral aminoaciduria in cystathionine beta-synthase-deficient mice; an animal model of homocystinuria.

Akahoshi N, Kamata S, Kubota M, Hishiki T, Nagahata Y, Matsuura T, Yamazaki C, Yoshida Y, Yamada H, Ishizaki Y, Suematsu M, Kasahara T, Ishii I.

Am J Physiol Renal Physiol 306(12):F1462-76, 2014.

The authors utilized a mouse model for homocystinuria in order to examine renal amino acid reabsorbtion. Some of the immunohistochemistry experiments used anti-Met (Cat. #AB-T036). It was found that loss of cystathionine β-synthase causes hyperexcretion of both glucogenic and ketogenic neutral amino acids, as well as histidine.

TRPV1 expression level in isolectin B4-positive neurons contributes to mouse strain difference in cutaneous thermal nociceptive sensitivity.

Ono K, Ye Y, Viet CT, Dang D, Schmidt BL.

J Neurophysiol jn.00973.2014, 2015.

In order to determine whether IB4-positive trigeminal sensory neurons affect pain sensitivity, the authors administered 2 µg of rIB4-SAP (Cat. #IT-10) to the right infraorbital foramen. Saporin (Cat. #PR-01) was used as a control.

Macrophages are needed in the progression of tuberculosis into lung cancer.

Li J, Pan Y, Zhang B, Chen Q.

Tumour Biol 2015.

Approximately 30% of lung carcinomas also have tuberculosis lesions. The authors investigated the potential link between inflammatory processes and cancer in the lung. Mice with established tuberculosis infections received weekly 20 µg tail vein injections of Mac-1-SAP (Cat. #IT-06) in order to eliminate macrophages. Six months later the mice receiving Mac-1-SAP had a significantly lower incidence of lung carcinoma than control animals.

Dual targeting NG2 and GD3A using Mab-Zap immunotoxin results in reduced glioma cell viability in vitro.

Higgins SC, Fillmore HL, Ashkan K, Butt AM, Pilkington GJ.

Anticancer Res 35(1):77-84, 2015.

Human glioma-derived cell lines were sequentially incubated with anti-NG2 and anti-GD3A coupled to Mab-ZAP (Cat. #IT-04) at 1 µg/ml and 5 µg/ml for 72 hours each. The combination therapy was significantly more effective than single therapy in eliminating the glioma cells.

Activation of the mouse primary visual cortex by medial prefrontal subregion stimulation is not mediated by cholinergic basalo-cortical projections.

Nguyen HN, Huppe-Gourgues F, Vaucher E.

Front Syst Neurosci 9:1, 2015.

Mice received 1 µg icv injections of mu p75-SAP (Cat. #IT-16) to eliminate NGFr-positive cells. The results indicate a link between the prelimbic and infralimbic cortices and the primary visual cortex.

Preliminary results from a phase I study of substance P-saporin in terminal cancer patients with intractable pain.

Frankel AE, Nymeyer H, Lappi DA, Higgins D, Ahn C, Noe C.

J Clin Oncol 32 (suppl 31):191, 2014.

Existing pain therapies are insufficient to control cancer pain in 10-15% of patients. Substance P (SP) and its receptor, neurokinin-1 (NK-1r) have been determined to play a major role in spinal transmission of chronic pain. Animal studies have demonstrated that disruption of the NK-1r pathway alleviates chronic pain caused by a variety of stimuli. The authors are conducting a Phase I clinical trial in humans (NCT02036281) assessing the ability of SP-SAP to treat intractable chronic pain due to cancer. Patients have received intrathecal injections of 1, 2, or 4 µg of SP-SAP with no evidence of toxicity or neurological or cardiac abnormalities. Doses will escalate up to 90 µg.

Targeting Talk: Product Q&A

Q: I ordered a targeted toxin. Will it come in powder form? How do I re-dissolve it?

A: Our Saporin conjugate products are all provided in sterile PBS solution within a concentration range of 0.5 - 3 mg/ml. Saporin is an extremely safe 'toxin' to handle in standard laboratory environments when in solution for several reasons. Solutions in general are easier to corral and keep contained than powders and consequently are less likely to accidentally end up on an individual's skin, tongue, or in one's eyes. As a lyophilized product, Saporin would also be present at an extremely high concentration such that there is cause for concern should it contact the body of the user in any way. Lastly, our Saporin conjugates have historically required dilution prior to use for both *in vitro* and *in vivo* procedures. As such, it is much easier to ensure the amount of material you, as a customer, are receiving and the subsequent dilution is accurately adjusted to your desired concentration when providing these products already in solution. If upon receiving a Saporin conjugate you believe the product to be lyophilized or in a powder form, please contact us immediately, prior to opening the vial.

Q: I'm interested in your anti-DBH-saporin toxin for lesioning central catecholaminergic neurons. I see from the product description that the antibody used is a mouse monoclonal -- designed to specifically target rat DBH. My interest is to produce targeted lesions in mouse transgenic. Will this product still work specifically? Thanks.

A: Unfortunately, we do not have really good data to support the use of our Anti-DBH-SAP (Cat. #IT-03) in mice. There is significant homology between mouse and rat DBH, however the actual antigen for both the mouse monoclonal we use in the immunotoxin and an alternate unpurified rabbit polyclonal, is native bovine DBH enzyme. For further background information there are two references where our product was used in mice. The reference summaries from previous issues of *Targeting Trends* are listed below.

An early sympathetic nervous system influence exacerbates collagen-induced arthritis via CD4+ / CD25+ cells.[1] The sympathetic nervous system can play conflicting roles in collagen-induced arthritis (CIA). CD4+CD25+ T cells can play an immunoregulatory effect in this system depending on the expression of the FoxP3 transcription factor. Mice received 5-µg intraperitoneal injections of anti-DBH-SAP to induce an early sympathectomy. The results indicate that the sympathetic nervous system increases disease severity in CIA by stimulating some of the proinflammatory aspects of CD4+CD25+ T cells.

An opposing time-dependent immune-modulating effect of the sympathetic nervous system conferred by altering the cytokine profile in the local lymph nodes and spleen of mice with type II collagen-induced arthritis.[2] In this work the authors examined the role of the sympathetic nervous system (SNS) in late stages of chronic arthritis. 5 µg intraperitoneal injections of anti-DBH-SAP in mice were used to confirm that previous 6-OHDA injections caused a sympathectomy. The results demonstrate that the SNS supports inflammation during the asymptomatic phase of arthritis, but inhibits inflammation during the chronic symptomatic phase.

1. Harle P, Pongratz G, Albrecht J, Tarner IH, Straub RH *Arthritis Rheum* 58(8):2347-2355, 2008.
2. Harle P, Mobius D, Carr DJ, Scholmerich J, Straub RH *Arthritis Rheum* 52(4):1305-1313, 2005.

Q: Recently we used your flow cytometry services (Cytometry Research, ATS subsidiary). Based on post flow data analysis needs, I am providing the assay group list below (withheld for confidentiality).

A Yes, all these antibodies meet the requirement of being excited by our 488nm laser, however the specific combinations you list use fluorescent probes that have emission wavelengths that are too similar and would actually be detected on the same fluorescent channel. In essence, you would be unable to differentiate which target was being detected.

Here are suggestions for common combinations of commercially available fluorescent probes if you are interested in analyzing three colors simultaneously. All these probes can be excited at 488nm and will work on our equipment, so just make sure you don't have more than one probe from each channel.

FL1 Channel	FL2 Channel	FL-3 Channel
Alexa Fluor 488	Phycoerythrin (PE)	PE/Cy5
DyLight 488	Cy3	PE/Cy5.5
FITC		PerCP
GFP		PerCP/Cy5.5
		PE/Cy7

Drug-free selection of stable transfectants using rIB4-SAP

(continued from page 1)

mammalian cells, except in humans and Old World monkeys.

Transient expression of EndoGalC should lead to resistance in some transfected cells to isolectin BS-I-B_4 conjugated to saporin (rIB4-SAP; #IT-10; Advanced Targeting Systems Inc.), which causes cell death of α-Gal epitope-expressing untransfected cells, mainly because rIB4-SAP, internalized via specific binding to the cell-surface α-Gal epitope, inhibits protein synthesis.[1,2] During the period (~3 days after transfection) of transient expression of exogenous DNA (pCAG/EndoGalC), expression of α-Gal epitope on the cell surface is lost, allowing the cell to survive rIB4-SAP treatment (as schematically depicted in Fig. 1). Concomitantly, the co-introduced plasmid carrying the GOI has a chance to be integrated into host chromosomes. Untransfected cells continue to express the α-Gal epitope, which is specifically recognized by rIB4-SAP, and are eliminated after about 10 days of cultivation (Fig. 1). Thus, the surviving cell population is expected to express the GOI and the α-Gal epitope, since pCAG/EndoGalC introduced into a cell is lost during the 10-day cultivation after rIB4-SAP treatment. This concept has been previously explored by us,[3] in which we demonstrated that the GOI could be effectively integrated into host chromosomes via the *piggyBac* system after rIB4-SAP treatment.

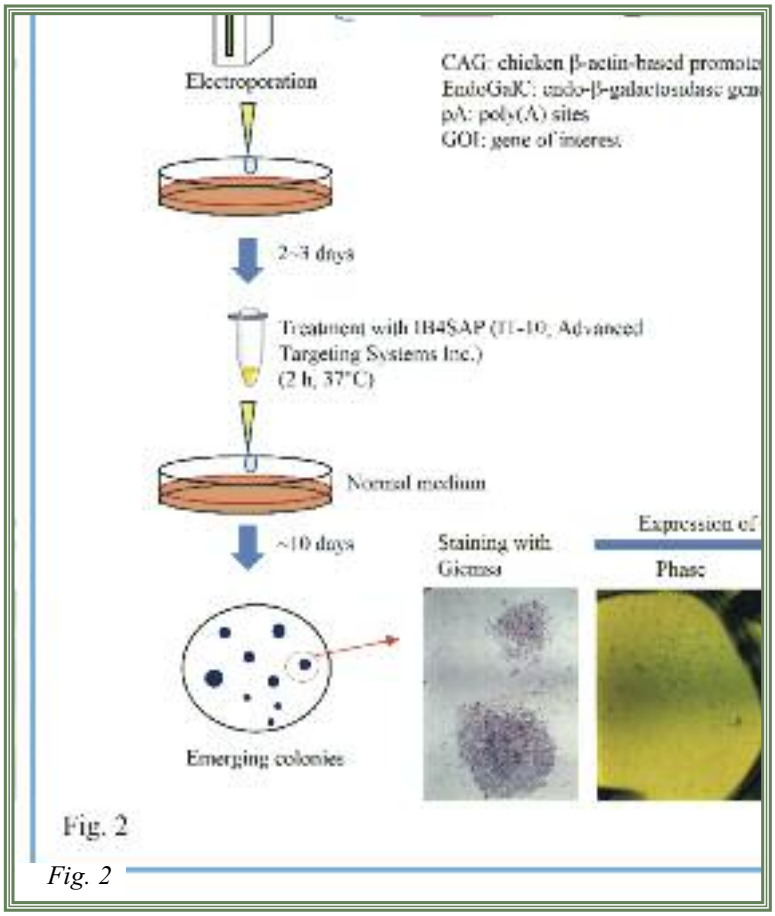

Fig. 2

Fig. 2

This drug-free acquisition of stably transfected cells is a very simple and convenient system. We prepared only two vectors, an EndoGalC-expression vector (in circular form) and a vector (in linearized form) carrying a GOI (Fig. 2). These vectors were mixed with trypsinized porcine fetal fibroblasts and were then delivered to the cells via electroporation. Immediately after electroporation, all transfected cells were seeded onto a dish containing normal medium and cultured for 2–3 days. The cells were then trypsinized and treated with 80 μg/mL rIB4-SAP in a 0.5-mL microfuge tube for 2 h at 37°C. Then, these cells were seeded in normal medium and cultured for ~10 days until colonies developed. The colonies were stained with Giemsa and were seen to express the GOI (tdTomato), as shown in the images at the bottom of Fig. 2. This system does not require selective drugs such as G418. Therefore, it does not require a pilot study to test the effectiveness of the drugs using untransfected cells. Furthermore, it will be useful for gene delivery to cells that are resistant to several selective drugs.

References

1. Akasaka E, Watanabe S, Himaki T, Ohtsuka M, Yoshida M, Miyoshi K, Sato M. (2010) Enrichment of xenograft-competent genetically modified pig cells using a targeted toxin, isolectin BS-I-B4 conjugate. *Xenotransplantation* 17(1):81-89.
2. Sato M, Akasaka E, Saitoh I, Ohtsuka M, Nakamura S, Sakurai T, Watanabe S. (2013) Targeted toxin-based selectable drug-free enrichment of Mammalian cells with high transgene expression. *Biology (Basel)* 2(1):341-355.
3. Sato M, Inada E, Saitoh I, Matsumoto Y, Ohtsuka M, Miura H, Nakamura S, Sakurai T, Watanabe S. (2015) A combination of targeted toxin technology and the piggyBac-mediated gene transfer system enables efficient isolation of stable transfectants in nonhuman mammalian cells. *Biotechnol J* 10(1):143-153.

Targeting Tools: Custom Biotinylation Service

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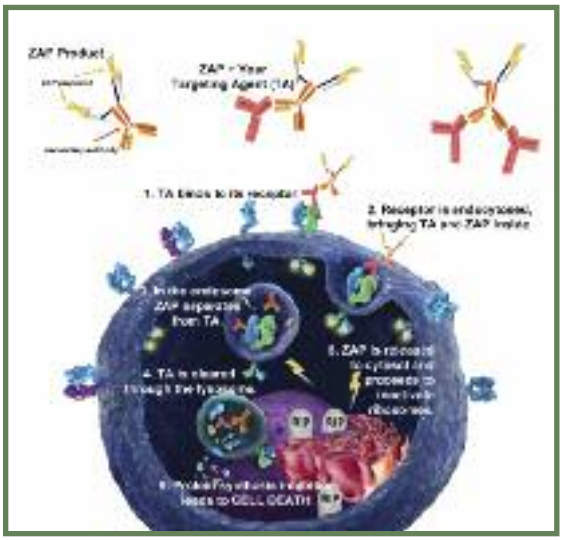
ONLY reacts with free amine groups	Discuss options available for your protein
	Includes Biotin-Z Internalization Kit
	Zero Hands-On time for you

*Dependent upon peptide, but typically available for most proteins such as antibodies



The streptavidin-biotin interaction has found extensive use as a research tool. The bond created between streptavidin and biotin is rapid and essentially non-reversible, unaffected by most extremes of pH, organic solvents, and denaturing reagents. A variety of molecules, including lectins, proteins, and antibodies, can be biotinylated and reacted with streptavidin labeled probes or other detection reagents for use in biological assays.

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The key component of the Biotin-Z Internalization Kit is Streptavidin-ZAP; Streptavidin chemically attached to Saporin, the most potent (and safest for use in the laboratory) of the plant ribosome-inactivating proteins. Streptavidin-ZAP "piggybacks" onto YOUR biotinylated material in order to evaluate the ability of the reagent to internalize upon binding to its receptor. Using Streptavidin-ZAP and biotinylated targeting agents, specific cytotoxins can be created JUST BY MIXING!

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- Average chemical ratio of moles of biotin to moles of protein
- Average effective molecular weight

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§ or anything recognized on the cell surface and internalized.

The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.

Cells that do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles **taken from last quarter's cover story**, one letter to each block, to solve the puzzle.

INSTOPPERY

EXCLOMP

STARVERE

COLOTOMONI

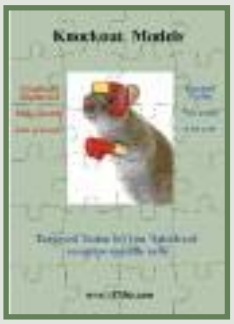
REIGNCOLICH

How using saporin conjugates affects the scientist's New Year.

Arrange the circled letters to form the answer, as suggested by the above clue.

ANSWER:
IT KEEPS RESEARCH RIGHT ON . . . ▢▢▢▢▢▢ !

WIN!



SOLVE the puzzle online with the correct solution by April 30, 2015.

WIN a ATS jigsaw puzzle!

www.atsbio.com/news/15q2_teaser.html

Jul-Aug-Sep 2015
Volume 16, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

SP-SAP Human Clinical Trial for Cancer Pain

An Anesthesiologist's Point of View

by Carl Noe, M.D., Anesthesiology & Pain Management, UT Southwestern Medical Center, Dallas, TX; and Eugene McDermott Center for Pain Management, Dallas, Texas

Dr. Noe is the Principal Investigator in the ongoing trial of SP-SAP

Intrathecal SP-SAP (Substance P attached to the ribosome-inactivating protein, saporin) has been studied in a Phase 1 clinical trial of patients with cancer pain at doses of 1, 2, 4, 8, 16 and 32 mcg. The first patient was treated April 10, 2014. Doses of 64 mcg and 90 mcg remain in Phase 1a of the clinical protocol. Phase 1b will treat multiple patients at the most effective dose. To date, no toxicity has been observed and the study is ongoing to evaluate response, safety, and tolerability. (For information about the trial, please visit: http://clinicaltrials.gov/show/NCT02036281).

SP-SAP is administered via an intrathecal catheter placed in the lumbar spine with the use of fluoroscopy and radiopaque contrast injection to ensure accurate delivery of the active drug. So far, the catheter placement has been at L5 vertebral level. The same location was used in the veterinary trial conducted by Dr. Dottie Brown in dogs with osteosarcoma.[1]

The lack of side effects or toxicity have led the clinical trial team to consider possible changes to the protocol that would enhance the effectiveness of SP-SAP in treating chronic pain. A primary endpoint of the trial is: response as defined by a 20% reduction in chronic pain or opioid dose within 4 weeks of treatment. One of the patients has clearly met this endpoint with reduction of pain medication by >20% during a 4-week period, following SP-SAP treatment.

Discussions are ongoing regarding several relevant issues that may affect efficiency of drug delivery and efficacy. 1) Modification of catheter placement may produce more selective responses and reduce required doses.

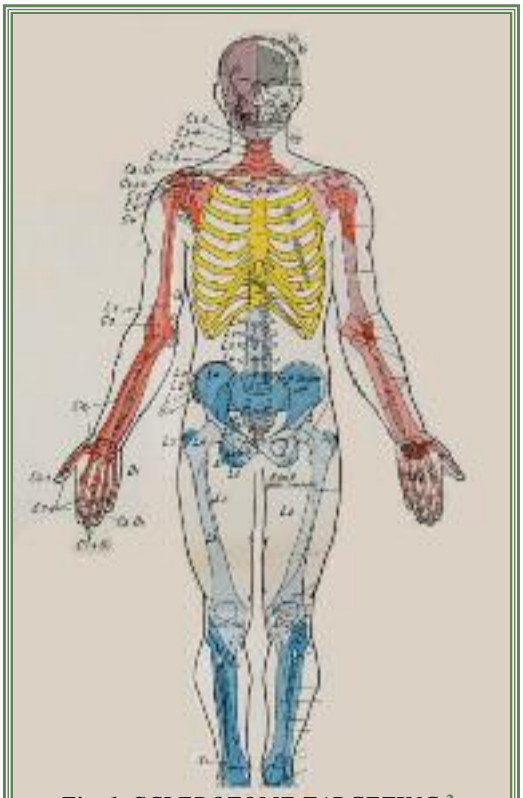


Fig. 1 SCLEROTOME TARGETING [2]
The sclerotome is the area of a bone innervated from a single spinal segment.

2) Targeting specific spinal segments using sclerotomes (Fig. 1) may be useful in delivering SP-SAP to a spinal cord location related to the pain origination. Cancer pain may be localized primarily to a bone; in these cases, using a sclerotomal map may help guide therapy to a specific nerve root and spinal cord location.

(continued on page 6)

Inside this issue:

Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Product Managers Highlight Their Products

MATTHEW KOHLS

While recombinant IB4-SAP (rIB4-SAP, Cat. #IT-10) has traditionally been used to eliminate cell populations that display alpha-D-galactose on the cell membrane, such as non-peptidergic c-fiber nociceptor neurons, it has also been found to be a very effective way to create stable transfected cell lines without the use of drug resistance genes. A recent publication by Sato *et al.* (*J Biotechnology* 10(1):143-153, 2015) demonstrates a new use for this conjugate.

LEONARDO ANCHETA

Looking for a new way to use our targeted toxins? Let ATS shed a little light on the subject. Researchers have used our targeted toxin technology in conjunction with photochemical internalization (PCI), a light-triggered technique that can help facilitate release of molecules from endocytic vesicles once inside the cytosol. Researchers are using this method to overcome resistance that develops towards therapeutic agents or intracellular barriers encountered when introducing molecules into cancer cells. Berstad *et al*. (*Biochim Biophys Acta* 1820(12):1849-1858, 2012), Bostad *et al*. (*Mol Pharm* 11(8):2764-2776, 2014), and Berg *et al*. (*Methods Mol Biol* 635:133-145, 2010) demonstrate the use of this pairing with biotinylated antibodies combined with streptavidin-ZAP (Cat. #IT-27).

LUCAS CHANCE

No, he's not a Product Manager. . . yet!

Welcome to Lucas Ancheta (son to Leonardo and Kate Ancheta), born April 14, 2015, weighing in at 5 lb, 2 oz.

BRIAN RUSSELL

Opioid receptors are a group of inhibitory G protein-coupled receptors that are heavily involved in analgesia, respiratory depression, GI motility, and addiction. ATS has a comprehensive collection of products that help researchers illuminate the role of opioid receptors. Dermorphin-SAP (Cat. #IT-12), Deltorphin-SAP (Cat. #BETA-006), Dynorphin-SAP (Cat. #IT-68), and Nociceptin-SAP (Cat. #BETA-001) have been shown to selectively eliminate cells expressing the mu (MOR), delta (DOR), kappa (KOR), or the ORL1 receptor, respectively. With these tools in hand, scientists have continued to discover new ways in which animals, and by extension, humans, respond to exogenous opioid therapy.

PATRICK SHRAMM

Cannabinoid receptors are involved in a variety of physiological responses including appetite, pain sensation, mood, and memory. We are now offering Anti-CB1-SAP (Cat. #BETA-005) to target and eliminate cells that express the CB1 receptor. Take advantage of this targeted toxin while it's still available as a beta product. If you're the first to publish data* you'll receive a $500 credit for use on ATS products.

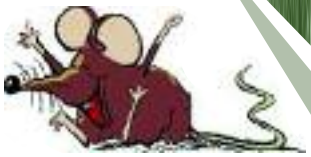
**Data subject to review.*

CHELSEA FRIEDMAN

Chlorotoxin, a peptide from the venom of the deathstalker scorpion, has been shown to bind to matrix metalloproteinase-2 (MMP-2) isoforms. These isoforms are upregulated in gliomas and other cancers of neuroectodermal origin, but are not normally expressed in the brain. Chlorotoxin-SAP (Cat. #BETA-010) is a bonded toxin between chlorotoxin and saporin, and can be a helpful tool in your research by allowing you to specifically target and eliminate cells expressing MMP-2 isoforms without affecting other cells.

Congratulations to the puzzle solvers from last quarter. Each winner will receive an ATS jigsaw puzzle.

Solve this quarter's teaser at www.ATSbio.com/news/15q3_teaser.html



LAST QUARTER'S WINNERS: Glenn H. Kageyama, Cal Poly Pomona Univ * Dave Ginsberg, Molecular Innovations * Judene Bliss, Roswell Park Cancer Institute * Daniel Pekala, Charles River Laboratories * Joan Schein, Biochain * Seto Chice, SUNY HSC at Brooklyn * Bill Henry, Rhode Island Hospital

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Targeted ablation of cholinergic interneurons in the dorsolateral striatum produces behavioral manifestations of Tourette syndrome.

Xu M, Kobets A, Du JC, Lennington J, Li L, Banasr M, Duman RS, Vaccarino FM, DiLeone RJ, Pittenger C.

Proc Natl Acad Sci U S A 112(3):893-898, 2015.

Postmortem studies of Tourette syndrome patients has revealed a reduction in the number of specific striatal interneurons. The authors explored the hypothesis that this neuronal deficit is enough to produce the symptoms of Tourette syndrome in mice. Animals received 90-ng injections of Anti-ChAT-SAP (Cat. #IT-42) into the striatum. Rabbit IgG-SAP (Cat. #IT-35) was used as a control. The data suggest that loss of the striatal interneurons is enough to produce some, but not all, of the symptoms caused by Tourette syndrome.

Role of striatal cholinergic interneurons in set-shifting in the rat.

Aoki S, Liu AW, Zucca A, Zucca S, Wickens JR.

J Neurosci 35(25):9424-9431, 2015.

The authors examined the role that cholinergic interneurons in the striatum play in a process called strategy set-shifting, where an attentional shift is required. Rats received bilateral injections of Anti-ChAT-SAP (Cat. #IT-42) into either the dorsomedial striatum or ventral striatum (500 ng total). Initial task learning was unaffected by either lesion. Lesioned animals displayed set-shifting deficits, and the deficit characteristics depended on the location of the lesion.

A central role for spinal dorsal horn neurons that express neurokinin-1 receptors in chronic itch.

Akiyama T, Nguyen T, Curtis E, Nishida K, Devireddy J, Delahanty J, Carstens MI, Carstens E.

Pain 156(7):1240-1246, 2015.

Chronic itch is caused by increased sensitivity of itch-signaling pathways. It can be generated by normally itchy stimuli (hyperknesis) and by normally non-itchy light touch (alloknesis). The authors used an ovalbumin-induced atopic dermatitis model to study chronic itch in mice. The mice received 400-ng intrathecal injections of Bombesin-SAP (Cat. #IT-40), SSP-SAP (Cat. #IT-11), or the control Blank-SAP (Cat. #IT-21). While Bombesin-SAP significantly attenuated hyperknesis, it had no effect on spontaneous scratching or alloknesis. SP-SAP reduced all behavioral signs of chronic itch.

Neurokinin 3 Receptor-Expressing Neurons in the Median Preoptic Nucleus Modulate Heat-Dissipation Effectors in the Female Rat.

Mittelman-Smith MA, Krajewski-Hall SJ, McMullen NT, Rance NE.

Endocrinology 156(7):2552-2562, 2015.

Kisspeptin and Neurokinin B (NKB) expression in the infundibular, or arcuate, nucleus is increased after menopause. Here the authors investigate whether KNDy (kisspeptin, NKB, and dynorphin expressing) neurons are able to influence cutaneous vasodilation through Neurokinin 3 (NK3)-expressing projections from the median preoptic nucleus (MnPO). Rats received two 10-ng injections of NK3-SAP (Cat. #IT-63) into the MnPO. Blank-SAP (Cat. #IT-21) was used as a control. The data indicate that NK3-expressing neurons in the MnPO facilitate vasodilation.

Hindbrain catecholamine neurons activate orexin neurons during systemic glucoprivation in male rats.

Li AJ, Wang Q, Elsarelli MM, Brown RL, Ritter S.

Endocrinology Epub2015.

Norepinephrine and epinephrine-secreting catecholamine neurons are strong stimulators of food intake. The authors investigated the interaction between these catecholamine neurons and orexin neurons in the perifornical lateral hypothalamus (PeFLH), which are known to be involved with the stimulation of food intake, increased arousal, and behavioral activation. Rats received 82-ng injections of Anti-DBH-SAP (Cat. #IT-03) into the PeFLH terminal field in order to lesion catecholamine neurons. Saporin (Cat. #PR-01) was used as a control. Assessment of food intake in response to 2-deoxy-D-glucose, as well as selective catecholamine activation, indicated that orexin neuron activation may be involved in glucoprivic appetite responses.

Orexin-A Enhances Feeding in Male Rats by Activating Hindbrain Catecholamine Neurons.

Li AJ, Wang Q, Davis H, Wang R, Ritter S.

Am J Physiol Regul Integr Comp Physiol Epub2015.

Although administration of orexin, norepinephrine, and epinephrine all induce significantly increased food intake, the potential interaction between the networks affected by these molecules has not been studied. In this work, the authors investigate the hypothesis that orexin neurons may stimulate feeding through the activation of catecholamine neurons. Rats received 82-ng injections of Anti-DBH-SAP (Cat. #IT-03) into the hypothalamus in order to lesion hypothalamically-projecting catecholamine neurons. Saporin (Cat. #PR-01) was used as a control. While the normal response to orexin A is increased food intake, lesioned animals did not display this response, indicating that catecholamine neurons are necessary for orexin modulation of food intake.

Selective optogenetic stimulation of the retrotrapezoid nucleus in sleeping rats activates breathing without changing blood pressure or causing arousal or sighs.

Burke PG, Kanbar R, Viar KE, Stornetta RL, Guyenet PG.

J Appl Physiol (1985) 118(12):1491-1501, 2015.

Hypoxia and hypercapnia both play roles in the activation of normal breathing. If either one is severe enough, arousal will also occur. The authors looked to better define the CNS pathways utilized by hypoxia and hypercapnia, as well as the pathways responsible for activation of arousal due to these conditions. The authors used optogenetic activation of the retrotrapezoid nucleus and C1 and A5 catecholaminergic

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

neurons, as well as selective C1 neuron stimulation in rats. Some rats also received bilateral injections of Anti-DBH-SAP (Cat. #IT-03) totaling 0.88 µg into the region of the lateral horn of the second thoracic segment.

Role of cerebrospinal fluid-contacting nucleus in sodium sensing and sodium appetite.

Xing D, Wu Y, Li G, Song S, Liu Y, Liu H, Wang X, Fei Y, Zhang C, Li Y, Zhang L.
Physiol Behav 147:291-299, 2015.

Sodium concentration in the cerebrospinal fluid (CSF) is tightly regulated, and this regulation requires numerous sensors spread throughout the brain. Here the authors injected 900 ng CTB-SAP (Cat. #IT-14) into the lateral ventricles. Investigation of spontaneous and induced sodium intake indicates the CSF-contacting nucleus is an important link in the sodium sensing network, and interacts with the lateral parabrachial nucleus.

Inflammatory Macrophages Promotes Development of Diabetic Encephalopathy.

Wang B, Miao Y, Zhao Z, Zhong Y.
Cell Physiol Biochem 36(3):1142-1150, 2015.

Diabetes can cause neuroinflammation leading to dementia. Diabetes was induced in mice by injection of streptozotocin (STZ). In order to investigate the role of inflammatory macrophages in the development of diabetic encephalopathy, the authors used twice weekly 20-µg IP injections of Mac-1-SAP (Cat. #IT-06). Mice receiving Mac-1-SAP had significantly reduced numbers of inflammatory macrophages in the brain, and also reduced responses to STZ injection.

Striatal patch compartment lesions reduce stereotypy following repeated cocaine administration.

Murray RC, Logan MC, Horner KA.
Brain Res Epub2015.

Stereotypy is defined as abnormally repetitive motor movements accompanied by an inability to initiate normal adaptive responses. Psychostimulants such as cocaine will often produce these movements. It is thought that stereotypy is related to activation of the patch compartment of the striatum. In order to better understand the function of the patch compartment in stereotypy due to repeated exposure to cocaine, the authors administered bilateral injections of Dermorphin-SAP (Cat. #IT-12) into the rostral striatum. Saporin (Cat. #PR-01) was used as a control.

Role of adrenomedullin in the cerebrospinal fluid-contacting nucleus in the modulation of immobilization stress.

Wu YH, Song SY, Liu H, Xing D, Wang X, Fei Y, Li GL, Zhang C, Li Y, Zhang LC.
Neuropeptides 51:43-54, 2015.

The CSF-contacting nucleus (CSF-CN) is a brain structure containing neurons that can bidirectionally transmit signals between the brain parenchyma and the CSF. In order to better understand what regulatory peptides modulate this organ, the authors eliminated the CSF-CN of rats with a 500-ng icv injection of CTB-SAP (Cat. #IT-14). Saporin (Cat. #PR-01) was used as a control. The elimination of the CSF-CN worsened the response to chronic immobilization stress; with other data this information suggests that the CSF-CN uses adrenomedullin as a stress-related peptide.

Treatment Considerations for Cancer Pain: A Global Perspective.

Pergolizzi JV, Gharibo C, Ho KY.
Pain Pract Epub2014.

This review discusses the treatment of cancer pain, addressing various aspects of the overall picture, such as early pain treatment to reduce central sensitization and chronic pain, pain assessment tools, and guidelines for treating specific populations of patients. Some of the current tools for pain management are discussed, including SP-SAP, which is currently in clinical trials as a cancer pain therapeutic (see cover article).

Don't see your publication here? Send us a PDF at ats@ATSbio.com and we'll be sure to review it in our next issue.

Novel Mechanisms of Spinal Cord Plasticity in a Mouse Model of Motoneuron Disease.

Gulino R, Parenti R, Gulisano M.
Biomed Res Int 2015:654637, 2015.

Here the authors investigate spinal plasticity mechanisms involving a number of different proteins, including BDNF, Shh, Notch-1, Numb, and Noggin. The model used is a mouse motoneuron depletion strategy, where the animals receive 3 µg of CTB-SAP (Cat. #IT-14) into each of the medial and lateral gastrocnemius muscles. The results indicate that TDP-43, a nuclear DNA/RNA binding protein, may be an important regulator of synaptic plasticity.

Effects of immunotoxic and electrolytic lesions of medial septal area on spatial short-term memory in rats.

Dashniani M, Kruashvili L, Rusadze Kh, Mataradze S, Beselia G.
Georgian Med News (239):98-103, 2015.

In this work the authors investigated how essential septohippocampal projections are in a spatial working memory model. Rats received bilateral injections of 192-IgG-SAP (Cat. #IT-01, 600 ng total) or GAT-1-SAP (Cat. #IT-32, 195 ng total) into the medial septum. Saporin (Cat. #PR-01) was used as a control.

Selective lesion of gaba-ergic neurons in the medial septum by gat1-saporin impairs spatial learning in a water-maze.

Burjanadze M, Mataradze S, Rusadze K, Chkhikvishvili N, Dashniani M.
Georgian Med News (240):59-64, 2015.

The authors investigated the role of GABAergic neurons in the medial septum on spatial learning using a Morris water maze test. Rats received bilateral injections totaling 162 ng of GAT-1-SAP (Cat. #IT-32) into the medial septum. Saporin (Cat. #PR-01) was used as a control. The lesioned animals

(continued on page 5)

Targeting Talk: Product Q&A

Q: For in vitro cytotoxicity assays, could you tell me:
1) whether you incubate primary with your Saporin secondary for a specific amount of time prior to cell addition, and
2) do you use a single concentration of secondary per well or a primary:secondary ratio -- like 1:2 or 1:4?

A: The primary antibody should be incubated with the ZAP product for 20 min prior to addition to the cells. Internalization often happens so quickly that you would lose some efficacy due to the antibody being bound and internalized prior to the ZAP product complexing with the primary.

We do recommend maintaining a constant 5 nM (~ 45 ng/well) concentration of the ZAP product in the well and titrating your primary only. This way the EC_{50} you generate will be the EC_{50} of the primary antibody with all else held constant. The best starting concentration for your primary antibody is 10-100 nM in the well.

Q: Your targeted toxin kits come with different controls. I'm not sure of the best way to use them; there is included unconjugated antibody, unconjugated saporin, and a control conjugate, mouse IgG-SAP. Should I use them all in the same experiment or for different purposes?

A: For mouse IgG-containing conjugates, the ideal control is Mouse IgG-SAP (Cat. # IT-18). Mouse IgG-SAP — that is, saporin conjugated to mouse IgG — that has no specific antigen for targeting is the best control. Unconjugated saporin is still considered a second good control, useful in cases where down-regulation by the antibody is a concern.

Q: Which control is best to use with Octreotide-SAP?

A: The best control to use with Octreotide-SAP is Blank-SAP (Cat. #IT-21). Blank-SAP serves as a control for all our peptide-targeted SAP conjugates. Listed below are the appropriate controls to use with our primary saporin conjugates.

Send a message on our website to get answers to your targeting questions.

(continued from page 3)

displayed significant deficits during the water maze task, indicating that GABAergic neurons in the medial septum are intrinsic to organization of spatial map-driven behavior.

Exploratory behavior and recognition memory in medial septal electrolytic, neuro- and immunotoxic lesioned rats.

Dashniani MG, Burjanadze MA, Naneishvili TL, Chkhikvishvili NC, Beselia GV, Kruashvili LB, Pochkhidze NO, Chighladze MR.

Physiol Res Epub2015.

To investigate recognition memory that incorporates a spatial or temporal component, the authors lesioned the medial septum of rats using several techniques. For specific lesioning of cholinergic neurons rats received bilateral injections of 192-IgG-SAP (Cat. #IT-01, 500 ng total) into the medial septum. Saporin (Cat. #PR-01) was used as a control. While electrolytic lesions produced disruptions of spatial recognition memory, immunotoxin lesions did not, indicating that the cholinergic neurons of the septohippocampal pathway are not essential to processing this type of learning.

Appropriate Controls for Conjugates

Blank-CTA (IT-61)	peptide-targeted CTA conjugates	SP-CTA and Neurotensin-CTA
Blank-SAP (IT-21)	peptide-targeted SAP conjugates	SSP-SAP, MOR-SAP, CRF-SAP, NPY-SAP, CCK-SAP, Galanin-SAP, Bombesin-SAP, Oxytocin-SAP, Neurotensin-SAP, NK3-SAP, Dyno-SAP and NMB-SAP
Fab IgG-SAP (IT-67)	goat IgG Fab-ZAP secondary conjugates	Fab-ZAP mouse, Fab-ZAP human, Fab-ZAP rat, Fab-ZAP rabbit and FabFc-ZAP human
Goat IgG-SAP (IT-19)	goat IgG-containing targeted toxins	Mab-ZAP, Rab-ZAP, Hum-ZAP, Rat-ZAP, Anti-M-ZAP, Hug-M-ZAP and gPIG-ZAP
Human IgG-SAP (IT-49)	human IgG-containing targeted toxins	Custom Conjugates
Mouse IgG-SAP (IT-18)	mouse IgG-containing targeted toxins	192-IgG-SAP, OX7-SAP, Anti-DBH-SAP, ME20.4-SAP, Anti-SERT-SAP, Anti-CD25-SAP human, Mac-1-SAP rat, Anti-CD22-SAP, Anti-6 His-ZAP, Anti-GFP-ZAP, Anti-Basigin2-SAP, Anti-V5-ZAP, and Anti-FLAG (M5)-ZAP
Mouse IgM-SAP (IT-41)	mouse IgM-containing targeted toxins	Anti-M-ZAP
Rabbit IgG-SAP (IT-35)	rabbit IgG-containing targeted toxins	mu p75-SAP, GAT1-SAP, Goat-ZAP, Anti-ChAT-SAP, Melanopsin-SAP and Chick-ZAP
Rat IgG-SAP (IT-17)	rat IgG-containing targeted toxins	Mac-1-SAP, Anti-DAT-SAP, Anti-CD25-SAP mouse and Anti-CD103-SAP

SP-SAP Human Clinical Trial for Cancer Pain

(continued from page 1)

3) Targeting specific myotomes (Fig. 2). Patients with sarcomas may have pain in a soft tissue that can be localized to a myotome and the nerve root(s) that innervate the area. Using information from the patient, physical examination, imaging and myotomal charts may help target treatment. For example, suppose a patient has a sarcoma arising from myotome-derived skeletal muscle that is predominantly innervated by the left L2 nerve root. Theoretically, a catheter could be placed posterior to where the left L2 nerve root enters the cord so that the injected SP-SAP would be close to the dorsal horn (which is the target).

4) Consideration of the baricity of SP-SAP may also be useful to more efficiently direct the placement of the drug in the spinal fluid.

Along with discussions to improve drug delivery and efficacy are considerations of the patient population being treated now (end-stage cancer patients unresponsive to opioid treatment) and future populations that could benefit from treatment with SP-SAP. In the current trial, several patients had previous spine surgery that complicated the catheter placement for intrathecal treatment. Also, patients have had heterogeneous and progressive disease and worsening pain during the study, complicating the interpretation of responses. For example, several patients reported a reduction in opioid requirements and transient pain relief. In a population where pain continues to spread along with the cancer, it is difficult to determine if the transience is due to the dose level of SP-SAP (too little) or the establishment of 'new' pain.

End-stage cancer patients are a needy population that desperately need relief from chronic pain. The early signs of efficacy for SP-SAP are encouraging and the next doses (64 mcg and 90 mcg) may bring the long-term pain relief needed.

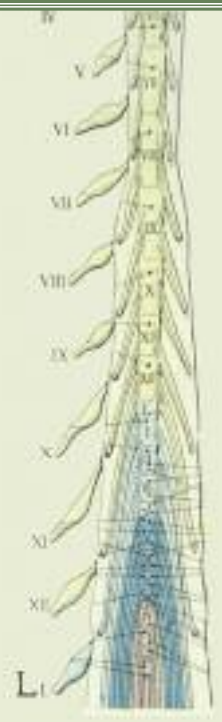


Fig. 2 MYOTOME TARGETING [2]
Each muscle in the body is supplied by both a particular level of the spinal cord and by its corresponding spinal nerve. A myotome is made up of the muscle and its nerve.

References

1. Brown DC, Agnello K. (2013) Intrathecal substance p-saporin in the dog: efficacy in bone cancer pain. Anesthesiology 119(5):1178-1185.
2. Sémiologie du système nerveux by Dejerine, J. (Joseph Jules), 1849-1917, Published 1901.

Upcoming Events

Society for Neuroscience
October 17-21, 2015
Chicago, IL
Booth #662

Pain & Migraine
Therapeutics Summit
September 23-24, 2015
Washington DC

Targeting Tools: Beta Products

Beta Products. Great Opportunity for Discovery at a Very Low Price.

Saporin conjugates specifically eliminate cells identified by an extracellular marker. Each week we release new products for beta testing, so check out the website frequently (http://atsbio.com/catalog/beta.php). There are two conjugate configurations for Beta products. The first is a **direct conjugate** of the targeting agent to saporin; the second is a **bonded toxin** between the targeting agent and the secondary conjugate Streptavidin-ZAP (Cat. #IT-27; saporin attached to streptavidin).

Each of the Beta products has:

- Saporin activity confirmed
- Peptide sequences and/or Antibody binding specificity published/confirmed

Nociceptin-SAP (Cat. #BETA-001)
bonded toxin • targeting agent: nociceptin

Octreotide-SAP (Cat. #BETA-002)
bonded toxin • targeting agent: octreotide peptide

Azido-ZAP (Cat. #BETA-003)
direct conjugate • targeting agent: terminal azide group

Anti-CB1-SAP (Cat. #BETA-005)
bonded toxin • targeting agent: Ab to hum/rat cannabinoid receptor 1

Deltorphin-SAP (Cat. #BETA-006)
direct conjugate • targeting agent: deltorphin

Anti-OX1r-SAP (Cat. #BETA-007)
bonded toxin • targeting agent: antibody to mouse, rat, guinea pig orexin 1 receptor

Anti-OX2r-SAP (Cat. #BETA-008)
bonded toxin • targeting agent: antibody to rat orexin 2 receptor

Exenatide-4-SAP (GLP-1-SAP; Cat. #BETA-009)
bonded toxin • targeting agent: exendin-4 peptide, a glucagon-like peptide-1 agonist

Chlorotoxin-SAP (Cat. #BETA-010)
bonded toxin • targeting agent: chlorotoxin

CGRP-SAP (Cat. #BETA-011)
bonded toxin • targeting agent: a-CGRP

Methotrexate-SAP (Cat. #BETA-012)
direct conjugate • targeting agent: Methotrexate

MOA-SAP (Cat. # BETA-013)
bonded toxin • targeting agent: blood group B lectin from Marasmius Oreades

Anti-PD-L1-SAP (Cat. #BETA-014)
bonded toxin • targeting agent: antibody to human PD-L1

Anti-CD105-SAP (Cat. #BETA-015)
bonded toxin • targeting agent: antibody to human CD105

Anti-CD38-SAP (Cat. #BETA-016)
bonded toxin • targeting agent: antibody to mouse CD38

Anti-Cripto-SAP (Cat. #BETA-017)
bonded toxin • targeting agent: antibody to mouse/human Cripto-1

Anti-CXCR4-SAP (Cat. #BETA-018)
bonded toxin • targeting agent: antibody to human CXCR4

Anti-MC4R-SAP (Cat. #BETA-020)
bonded toxin • targeting agent: antibody to human MC4R

ACTH-SAP (Cat. #BETA-021)
bonded toxin • targeting agent: ACTH peptide

αMSH-SAP (Cat. #BETA-022)
bonded toxin • targeting agent: αMSH peptide

Vasopressin-SAP (Cat. #BETA-023)
bonded toxin • targeting agent: vasopressin peptide

Anti-CD15-SAP (Cat. #BETA-024)
bonded toxin • targeting agent: antibody to CD15

Anti-CD24-SAP (Cat. #BETA-025)
bonded toxin • targeting agent: antibody to human CD24

VIP-SAP (Cat. #BETA-027)
bonded toxin • targeting agent: Vasoactive intestinal peptide

GnRH-SAP (Cat.#BETA-028)
bonded toxin • targeting agent: GnRH peptide

Kermit lounging comfortably.

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ATS binds SAPORIN with your ANTIBODY to make a powerful targeting agent.

§ or anything recognized on the cell surface and internalized.

The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.

Cells that do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles **taken from this issue's cover story**, one letter to each block, to solve the puzzle.

MOLESCROTE

CHEATTER

BRUMAL

DULIF

VACATINITING

What the students did when school was out for the summer.

Arrange the circled letters to form the answer, as suggested by the above clue.

ANSWER: THEY STUDIED THE . . . ◯◯◯◯◯◯◯ OF ◯◯◯◯◯◯◯◯ !

WIN!

SOLVE the puzzle online with the correct solution by September 30, 2015.

WIN a $100 product credit!

www.atsbio.com/news/15q3_teaser.html

Oct-Nov-Dec 2015
Volume 16, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

A specific immunotoxin elucidates a causal role of striatal cholinergic system in behavioral flexibility

by Sho Aoki and Jeffery R. Wickens

Neurobiology Research Unit, Okinawa Institute of Science and Technology, 1919-1 Tancha, Onna, Kunigami, Okinawa 904-0495, Japan

Behavioral flexibility is broadly defined as the ability to change behavioral strategy, according to a change of governing rules. Accumulated evidence suggests the involvement of particular brain areas such as prefrontal cortex and striatum in this function, in which specific brain regions play their own roles. An extension of understanding on neural substrates mediating behavioral flexibility needs a next step beyond the specificity of brain regions: the specific role of different neuronal subtypes. A method utilizing specific neurotoxins enabled us to target and elucidate the role of neurochemically-specific neurons in this ability. In our recent study,[1] we demonstrated a causal role of rat cholinergic interneurons in the striatum in behavioral flexibility, using a new specific immunotoxin targeting neurons containing choline acetyltransferase (ChAT). Comparing non-selective neuronal labeling and specific immunostaining of ChAT neurons indicated that local injections of the immunotoxin successfully and selectively damaged cholinergic neurons (Fig. 1). This result is consistent with a previous study that used Anti-ChAT-SAP to study the medial prefrontal cortex (Cat. #IT-42).[2]

Using the selective lesion, we compared intact rats injected with saline and rats without cholinergic interneurons of either dorsomedial or ventral striatum in a set-shifting task.[3] This task required animals to shift their attention from one stimulus dimension to

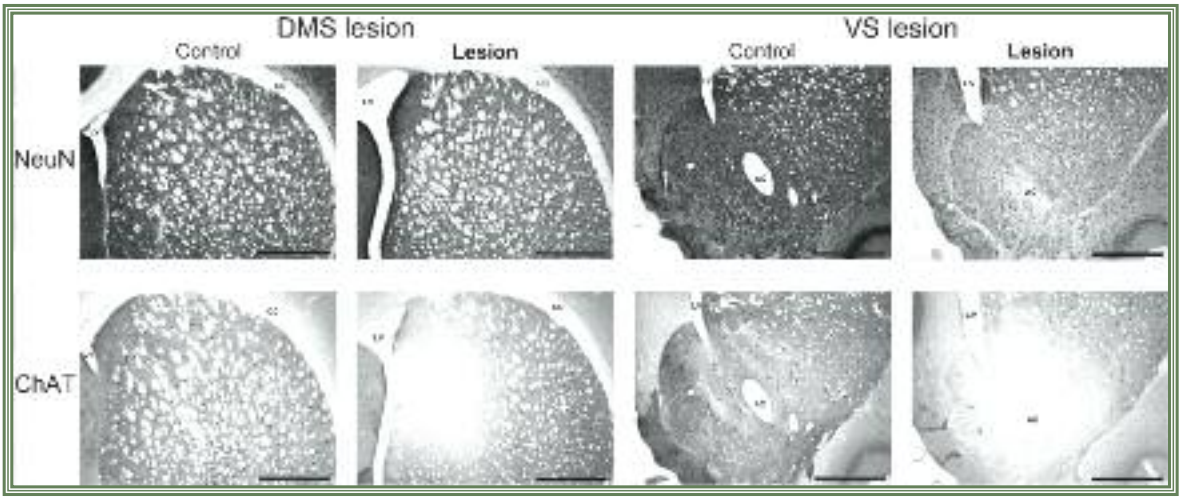

Fig. 1: Representative coronal sections of the rat striatum show intact nuclei (NeuN) staining but clear ablation of the cholinergic interneurons with ChAT staining in lesioned cases (DMS or VS). Abbreviations; DMS: dorsomedial striatum, VS: ventral striatum, LV: lateral ventricle, CC: corpus callosum, AC, anterior commissure. Scale bar: 1 mm. Reprinted from Aoki *et al.* (2015).

(continued on page 6)

Inside this issue:

Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

New Product: Mono-Biotin Saporin

The conjugation specialists at Advanced Targeting Systems are proud to announce a new addition to the catalog:

Mono-Biotin Saporin
Cat. #BT-PR01

Researchers now have more freedom in selecting conjugation strategies. Users have taken advantage of the ability to use biotinylated targeting agents with Streptavidin-ZAP (Cat. #IT-27) a chemical conjugate between saporin and streptavidin. Streptavidin-ZAP converts biotinylated materials into targeted toxins. Streptavidin is a tetrameric protein (molecular weight 53 kDa in its recombinant form), with each subunit able to bind a single biotin molecule. The bond between streptavidin and biotin is rapid and essentially non-reversible, unaffected by most extremes of pH, organic solvents, and denaturing reagents. It is the strongest known noncovalent biological interaction ($Ka = 10^{15}\ M^{-1}$) between protein and ligand. The streptavidin used to make Streptavidin-ZAP contains no carbohydrate group and has a neutral isoelectric point, which therefore reduces the nonspecific binding as compared to avidin. A variety of molecules, including lectins, proteins, and antibodies, can be biotinylated and reacted with streptavidin-labeled probes or other detection reagents for use in biological assays.

Now, there is **Mono-Biotin Saporin**, a chemical conjugate between saporin and biotin. Each lot is specifically manufactured and analyzed to have an average molecular ratio of **one biotin per one saporin**. The assurance of consistency as well as the controlled labeling potential between lots makes this a valuable new research tool. Use this new tool *in vitro* or *in vivo* with your targeting agent to broaden your targeted toxin possibilities.[1]

Saporin is a ribosome-inactivating protein, molecular weight 30 kDa, from seeds of the plant *Saponaria officinalis*.

- ***Saporin is safe for laboratory use under normal safety conditions***
- ***LD_{50} in mice is 4 mg/kg***
- ***Saporin does not have a method of cell entry on its own***

Reference

Minami SS, Sun B, Popat K, Kauppinen T, Pleiss M, Zhou Y, Ward ME, Floreancig P, Mucke L, Desai T, Gan L. (2012) Selective targeting of microglia by quantum dots. *J Neuroinflammation* 9:22.

Upcoming Events

Society for Neuroscience
October 17-21, 2015
Chicago, IL
Booth #662

Experimental Biology
April 2-6, 2016
San Diego, CA
Booth #TBA

Targeting Teaser Solution

The solution to the puzzle was:

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What the students did when school was out for the summer.

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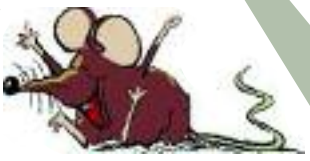

LAST QUARTER'S WINNERS: Seto Chice, SUNY HSC at Brooklyn * Corinne Kiessling, Binghamton University, NY

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Alterations in the rostral ventromedial medulla after the selective ablation of mu-opioid receptor expressing neurons.

Harasawa I, Johansen JP, Fields HL, Porreca F, Meng ID.

Pain Epub2015.

The rostral ventromedial medulla (RVM) has both excitatory and inhibitory control over nociceptive neurons in the medullary dorsal horn and spinal cord. Previous work has demonstrated that elimination of mu-opioid receptor-expressing neurons in the RVM reduces stress and injury-induced behavioral hypersensitivity, but the effect of losing these cells on the descending inhibitory system has not been examined. The authors administered 1.2 pmol of Dermorphin-SAP (Cat. #IT-12) to each side of the RVM of rats. Saporin (Cat. #PR-01) was used as a control. Characterization of RVM neurons in lesioned animals showed a reduction in on- and off-cells, but no change in the number of neutral cells. These data indicate that mu-opioid receptor-expressing cells in the RVM are not needed for analgesia produced by activation of RVM neurons.

CD103+ Dendritic Cells Elicit CD8+ T Cell Responses to Accelerate Kidney Injury in Adriamycin Nephropathy.

Cao Q, Lu J, Li Q, Wang C, Wang XM, Lee VW, Wang C, Nguyen H, Zheng G, Zhao Y, Alexander SI, Wang Y, Harris DC.

J Am Soc Nephrol Epub2015.

Although it is known that dendritic cells (DCs) are involved in chronic kidney disease, it is not well understood how they either resolve or aggravate the condition. CD103+ dendritic cells in particular, are known to maintain tolerance through interaction with regulatory T cells, as well as protect against infection through interactions with CD8+ T cells. In this work the authors depleted CD103+ DCs by administering 1 mg/kg of anti-CD103-SAP (Cat. #IT-50) to the intraperitoneal space of mice subject to adriamycin nephropathy. Rat IgG-SAP (Cat. #IT-17) was used as a control. Elimination of the CD103+ DCs attenuated the kidney injury, indicating that in murine chronic kidney disease CD103+ DCs are pathogenic rather than therapeutic.

Anti-EFNA4 Calicheamicin Conjugates Effectively Target Triple-Negative Breast and Ovarian Tumor-Initiating Cells to Result in Sustained Tumor Regressions.

Damelin M, Bankovich A, Park A, Aguilar J, Anderson W, Santaguida M, Aujay M, Fong S, Khandke K, Pulito V, Ernstoff E, Escarpe P, Bernstein J, Pysz M, Zhong W, Upeslacis E, Lucas J, Lucas J, Nichols T, Loving K, Foord O, Hampl J, Stull R, Barletta F, Falahatpisheh H, Sapra P, Gerber HP, Dylla SJ.

Clin Cancer Res 21(18):4165-4173, 2015.

Triple-negative breast cancer (TNBC) is characterized by tumors lacking HER2, estrogen receptor, and progesterone receptor. TNBC has proved to be very difficult to treat, in large part because of the absence of consensus targets on the surface of the tumor cells. In this work the authors empirically established a set of surface markers associated with TNBC tumor initiating cells, as produced by patient-derived xenografts. Ephrin-A4 was selected as a therapeutic target, and a cell line transfected with the ephrin-A4 gene was challenged with two versions of biotinylated anti-ephrin-A4 coupled to Streptavidin-ZAP (Cat. #IT-27). Both the mouse monoclonal and the humanized antibodies reach an EC_{50} of 10 ng/ml, indicating that ephrin-A4 has promise as a therapeutic target for TNBC.

KNDy neurons modulate the magnitude of the steroid-induced luteinizing hormone surges in ovariectomized rats.

Helena CV, Toporikova N, Kalil B, Stathopoulos AM, Pogrebna VV, Carolino RO, Anselmo-Franci JA, Bertram R.

Endocrinology Epub2015.

Maturation and reproductive function in mammals is controlled by the kisspeptin neuropeptide. Kisspeptin modulates numerous systems within this framework including the mediation of positive and negative feedback effects of estradiol on luteinizing hormone (LH). In the rat, two kisspeptin neuronal populations exist; one in the anteroventral periventricular nucleus (AVPV), and the KNDy (kisspeptin/neurokinin B/dynorphin) neurons in the arcuate nucleus. In this work the authors examine the role of KNDy neurons in estradiol positive feedback effects by administering 10-ng bilateral injections of NK3-SAP (Cat. #IT-63) into the arcuate nucleus of rats. The results indicate that KNDy neurons use dynorphin to inhibit AVPV neurons, establishing a regulatory mechanism for the amplitude of steroid-induced LH surges.

Membrane associated cancer-oocyte neoantigen SAS1B/ovastacin is a candidate immunotherapeutic target for uterine tumors.

Pires ES, D'Souza RS, Needham MA, Herr AK, Jazaeri AA, Li H, Stoler MH, Anderson-Knapp KL, Thomas T, Mandal A, Gougeon A, Flickinger CJ, Bruns DE, Pollok BA, Herr JC.

Oncotarget Epub2015.

Ovastatin is a zinc matrix metallo-proteinase thought to play roles in sperm-egg interaction and the prevention of polyspermy in eutherians. This protein is not found in normal adult tissues, but is expressed by uterine carcinosarcomas. The authors investigated the possibility of targeting ovastatin as a tumor surface neoantigen for therapeutic purposes. SNU539 cells, a uterine malignant mixed Müllerian tumor-derived cell line, were challenged with 1 µM, 0.1 µM, and 0.01 µM rabbit polyclonal anti-ovastatin coupled to 5.42 nM Fab-ZAP rabbit (Cat. #IT-57). Rabbit IgG-SAP (Cat. #IT-35) was used as a control. The results indicate that for this form of uterine cancer, ovastatin is a viable therapeutic target.

Neuropsin (OPN5)-mediated photoentrainment of local circadian oscillators in mammalian retina and cornea.

Buhr ED, Yue WW, Ren X, Jiang Z, Liao HR, Mei X, Vemaraju S, Nguyen MT, Reed RR, Lang RA, Yau KW, Van Gelder RN.

Proc Natl Acad Sci U S A Epub2015.

Circadian clocks are found in most mammalian tissues. These clocks are

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

synchronized by the suprachiasmatic nuclei (SCN) in the brain. The local clock found in the retina does not require rods, cones, intrinsically photosensitive retinal ganglion cells, or the SCN. In order to determine what photopigments are responsible for local retinal photoentrainment, the authors used a candidate gene approach. For immunohistochemical studies on flat mount retinas they used a melanopsin antibody (Cat. #AB-N38) at a 1:1000 dilution. The data indicate that OPN5, also known as neuropsin, has a light-sensing function and is involved in retinal photoentrainment.

Retrograde transport is not required for cytosolic translocation of the B-subunit of Shiga toxin.

Garcia-Castillo MD, Tran T, Bobard A, Renard HF, Rathjen SJ, Dransart E, Stechmann B, Lamaze C, Lord M, Cintrat JC, Enninga J, Tartour E, Johannes L.

J Cell Sci 128(13):2373-2387, 2015.

Bacterial and plant toxins rely on various trafficking pathways to reach intracellular targets. Shiga and Shiga-like toxins have been found to be moved via vesicular transport through several subcellular structures on the way to the cytosol. Shiga toxin (STx) is the cause of hemolytic uremic syndrome, for which there is no effective treatment. In order to better understand the mechanisms of STx membrane translocation the authors used a custom conjugate of the receptor-binding B-subunit of STx (STxB) and saporin (Custom conjugation provided by Advanced Targeting Systems). *In vitro* assays demonstrated that STxB-SAP did not use retrograde transport to the Golgi complex in order to reach the cytosol. This information has relevance to antigen cross-presentation of antigen-presenting cells.

Catecholaminergic neurons projecting to the paraventricular nucleus of the hypothalamus are essential for cardiorespiratory adjustments to hypoxia.

King TL, Ruyle BC, Kline DD, Heesch CM, Hasser EM.

Am J Physiol Regul Integr Comp Physiol Epub2015.

Catecholaminergic neurons in the brainstem are known to be involved in cardiorespiratory control and to modulate sensory function. Some of the projections from these neurons are to the paraventricular nucleus (PVN), and are involved in cardiorespiratory and neuroendocrine responses to hypoxia. While data have shown the PVN-projecting neurons are activated by hypoxia, their function in this context is not known. In this work the authors bilaterally injected 42 ng of Anti-DBH-SAP (Cat. #IT-03) into the PVN of rats. Mouse IgG-SAP (Cat. #IT-18) was used as control. Respiratory measurements of the lesioned animals indicates that PVN-projecting catecholaminergic neurons are involved in peripheral and central chemoreflex and arterial oxygen levels during exposure to hypoxic stimuli.

Catecholaminergic neurons in the comissural region of the nucleus of the solitary tract modulate hyperosmolality-induced responses.

Freiria-Oliveira AH, Blanch GT, Pedrino GR, Cravo SL, Murphy D, Menani JV, Colombari DS.

Am J Physiol Regul Integr Comp Physiol Epub2015.

Body fluid homeostasis and cardiovascular regulation are thought to be at least in part controlled by noradrenergic A2 neurons found in the nucleus of the solitary tract (NTS). In this work the authors investigated the involvement of A2 neurons of the commissural NTS in arterial pressure, as well as several body fluid homeostasis parameters. Rats received 12.6-ng injections of Anti-DBH-SAP (Cat. #IT-03) into the commissural NTS. Mouse IgG-SAP (Cat. #IT-18) was used as a control. Lesioned animals displayed increased c-Fos expression in the hypothalamic paraventricular nucleus when treated with hypertonic NaCl, and increased arterial pressure. The data indicate that commissural NTS A2 neurons are essential for inhibitory mechanisms that reduce water intake and pressor response to an acute increase in plasma osmolality.

Limited changes in spinal lamina I dorsal horn neurons following the cytotoxic ablation of non-peptidergic C-fibers.

Saeed AW, Pawlowski SA, Ribeiro-da-Silva A.

Mol Pain 11(1):54, 2015.

For the most part nociceptive information is moved from the periphery to the spinal cord through small diameter primary afferents. One subclass of these afferents is further divided into peptidergic and non-peptidergic populations. The authors examined the role of the non-peptidergic afferents in normal nociception and pain, especially the aspect that in rat neuropathic and inflammatory pain models there is novel expression of neurokinin-1 receptors in some neurons normally devoid of this protein. Rats received 4.8-µg injections of rIB4-SAP (Cat. #IT-10) into the left sciatic nerve, over three injection sites. While the number of non-peptidergic neurons was significantly reduced, de novo expression of the neurokinin-1 receptor was not increased in lamina I pyramidal projection neurons.

Selective elimination of isolectin B4-binding trigeminal neurons enhanced formalin-induced nocifensive behavior in the upper lip of rats and c-Fos expression in the trigeminal subnucleus caudalis.

Oyamaguchi A, Abe T, Sugiyo S, Niwa H, Takemura M.

Neurosci Res Epub2015.

In adult rats non-peptidergic neurons and peptidergic neurons innervate different areas and layers of the lamina. It is thought that these two neuronal populations play different roles in nociceptive processing, but the specific function of each group is not well understood. In order to investigate peptidergic and non-peptidergic neurons in orofacial pain processing the authors injected the cisterna magna of rats with 2.9 µg of rIB4-SAP (Cat. #IT-10). Blank-SAP (Cat. #IT-21) was used as a control. The lesioned

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(continued on page 5)

Targeting Talk: Product Q&A

Q: Hello, I have used your Anti-DBH-SAP (Cat. #IT-03) conjugate, and I'm having a hard time finding this citation: R.G. Wiley, D.A. Lappi. Suicide Transport and Immunolesioning. Molecular Biology Intelligence Unit, R.G. Landes Co, Austin, TX (1994). Do you know where I could find a copy?

A: This book is available in many university libraries and can also be purchased here: http://www.amazon.com/Suicide-Transport-Immunolesioning-Molecular-Intelligence/dp/1570590958

Q: I'm trying to find out if enough Anti-DBH-SAP will be retrogradely transported and taken up by non targeted sympathetic neurons by bulk fluid-phase endocytosis. Does saporin become degraded after it kills the neuron or does it enter the extracellular matrix?

A: It is very unlikely that a targeted toxin such as Anti-DBH-SAP is freed from the targeted neuron in a meaningful condition. There has never been a reported identification of a targeted toxin, functionally or not, after it has eliminated its targeted neuron. Current evidence indicates that effective suicide transport agents undergo endocytosis at nerve terminals followed by retrograde axonal transport of the endocytic vesicles containing the toxin. Experiments using vincristine have shown that the retrograde axonal transport of suicide transport toxins utilizes the fast transport system (microtubules). However, it is not known what determines whether or not a specific toxin-ligand undergoes axonal transport after internalization.

Empirically, it has been observed that immunotoxins (OX7-SAP [Cat. #IT-02], 192-IgG-SAP [Cat. #IT-01], Anti-DBH-SAP) and lectin-toxins (ricin, volkensin, IB4-SAP) all undergo retrograde axonal transport and are therefore effective suicide transport agents. This is not true, however, for neuropeptide-toxin conjugates, such as dermorphin-SAP. For example, in an unpublished study, we injected large doses (1-2 µg) of Dermorphin-SAP (Cat. #IT-12) into the lumbar intrathecal space of rats. After 2-3 days, rats were sacrificed and lumbar dorsal root ganglia examined for evidence of toxin effect (striking chromatolysis). None was found after examining numerous ganglia and >15,000 primary afferent neurons. Apparently, dermorphin-SAP is not retrogradely transported even if it is taken into the primary afferent terminals that express the mu opioid receptor (MOR).

(continued from page 4)

animals displayed more frequent face-rubbing responses on the administration of formalin, indicating that IB4-binding neurons in the trigeminal nerve play an anti-nociceptive role in response to this type of pain.

Hippocampal acetylcholine depletion has no effect on anxiety, spatial novelty preference, or differential reward for low rates of responding (DRL) performance in rats.

McHugh SB, Francis A, McAuley JD, Stewart AL, Baxter MG, Bannerman DM.
Behav Neurosci 129(4):491-501, 2015.

It is unclear whether cholinergic lesions in the hippocampus affect both learning and behavior, or learning only. In this study the authors lesioned cholinergic neurons in the medial septum/vertical limb of the diagonal band of Broca of rats with bilateral 30-ng injections of 192-IgG-SAP (Cat. #IT-01). Although hippocampal cholinergic innervations were significantly reduced, with a concomitant reduction in choline acetyltransferase activity, the lesioned animals did not perform differently in several behavioral tests. The data do not provide evidence that the septo-hippocampal cholingeric projections play a role in anxiety, spatial novelty preference, or differential reward for low rates of responding tests.

Selective C1 Lesioning Slightly Decreases Angiotensin II Type I Receptor Expression in the Rat Rostral Ventrolateral Medulla (RVLM).

Bourassa EA, Stedenfeld KA, Sved AF, Speth RC.
Neurochem Res Epub2015.

Exogenous angiotensin II administered to the RVLM produces a significant pressor response that can be countered by angiotensin II type I receptor antagonists. In this work the authors examined the relative contribution of C1 and non-C1 neurons in the RVLM to this angiotensin II response. Rats received 10 or 15 ng of Anti-DBH-SAP (Cat. #IT-03) as unilateral injections into the RVLM. Mouse IgG-SAP (Cat. #IT-18) was used as control. The data indicate that the majority of angiotensin II type 1 receptors are expressed on non-C1 neurons or glia.

Pain from intra-articular NGF or joint injury in the rat requires contributions from peptidergic joint afferents.

Kras JV, Weisshaar CL, Pall PS, Winkelstein BA.
Neurosci Lett 604:193-198, 2015.

Both peptidergic and non-peptidergic neurons innervate the facet joint, which is the source of pain in a majority of neck trauma. In this work the authors examined these subpopulations of neurons to determine the contribution of each in facet joint pain. 100 ng of SSP-SAP (Cat. #IT-11) was injected into bilateral C6/C7 facet joints of rats. Alternatively, rats received 5 µg of rIB4-SAP (Cat. #IT-10) via the same method. Saporin (Cat. #PR-01) was used as control. SSP-SAP, but not rIB4-SAP was able to prevent NGF-induced mechanical and thermal hypersensitivity. SSP-SAP administration also prevented behavioral hypersensitivity and NGF upregulation in the dorsal root ganglion after facet joint distraction. The data indicate that interference with peptidergic signaling within the facet joint may be a treatment for pain originating in that location.

Anti-ChAT-SAP elucidates a causal role in behavioral flexibility

(continued from page 1)

another to change action strategies, based on a change of behavioral rules. We extended an established task[3] by setting three experimental conditions for a set-shift (Fig. 2), all of which required a change between two strategies involving attention to different stimuli. In all the conditions, animals initially learned to obtain a reward by choosing a Right lever (Fig. 2, Response strategy). Subsequently, after the set-shift, animals faced a change of behavioral rules in which

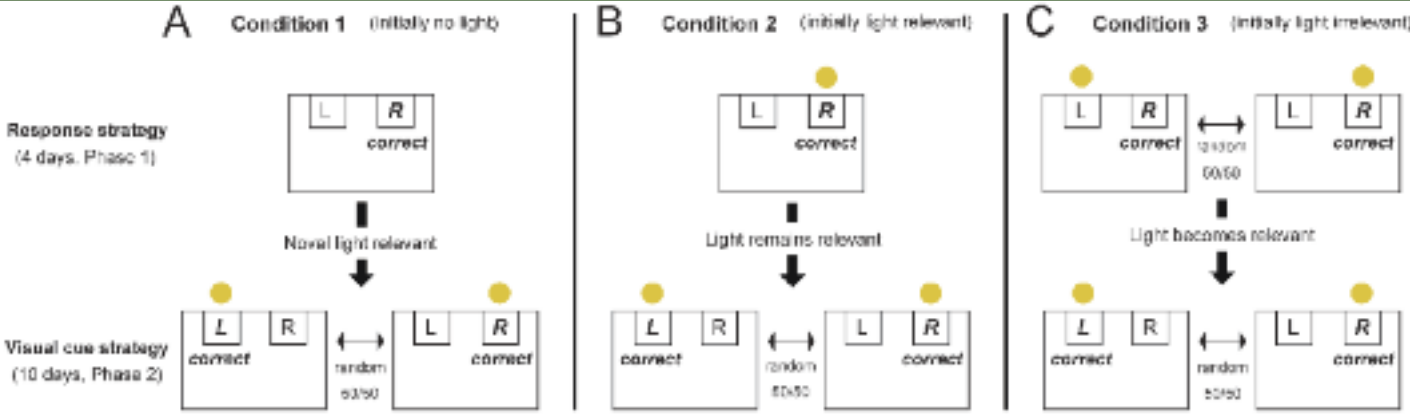

Fig. 2: A flow chart of three variations (A, B and C) of the set-shifting paradigm. A yellow circle shows a visual cue. Reprinted from Aoki *et al.* (2015).

animals had to learn to select a lever indicated by a light cue that randomly illuminated above either lever (Fig. 2, Visual cue strategy). Different manipulations of the light delivery in initial learning made it possible to test different attentional shifts in the next visual cue learning: attention to either 1) a previously absent but now novel light cue (Fig. 2A), 2) a previously relevant and remained relevant cue (Fig. 2B), and a previously irrelevant but now relevant cue (Fig. 2C).

Initial acquisition of response strategy was intact across conditions and treatments, indicating that the striatal cholinergic interneurons are unnecessary for initial learning. By contrast, after a change of behavioral rules occurred, both types of lesions made animals stick to an old strategy. They also showed less exploration for figuring a new rule out. Interestingly, ventral cholinergic ablation disrupted a strategic shift when it required attention to a novel light cue that was introduced as a new important stimulus (Fig. 2A). On the other hand, cholinergic loss in the dorsomedial striatum impaired a set shift when attention to a previously irrelevant cue was needed (Fig. 2C). There was no effect on a shift if the light remained relevant (Fig. 2B). These findings suggest that when facing a change of behavioral rules, striatal cholinergic interneurons play a specific role, namely inhibiting the use of an old strategy and facilitating exploration of a new rule. Furthermore, dorsomedial and ventral striatum cholinergic systems differentially contribute to this function in a highly context-dependent manner. Owing to the prominent targeting method by the Anti-ChAT-SAP, we found a causal role of a neurochemically-specific neuron in behavioral flexibility. This technique is undoubtedly powerful to deepen our knowledge of the causal relationship of particular neuronal types and behavior, and is encouraged for use in studies of different types of behavior.

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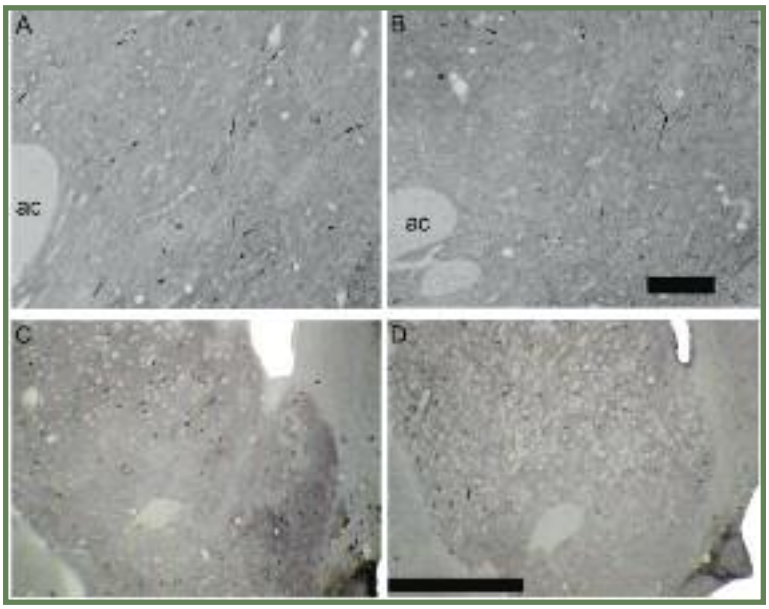
Targeting Tools: Featured Products

Anti-ChAT-SAP

Choline acetyltransferase (ChAT) catalyzes the synthesis of the neurotransmitter acetylcholine (ACh) from choline and acetyl-CoA in cholinergic neurons. ChAT serves as a specific marker for cholinergic neurons in both peripheral and central nervous systems. Evidence shows that ChAT exists in two forms inside cholinergic nerve terminals, a soluble hydrophilic form and the membrane-associated amphiphilic form.[1-2] Membrane-bound ChAT has served as the feature condition that allows specific targeting with an affinity-purified antibody to ChAT conjugated to saporin to specifically target and eliminate those specific cells. Anti-ChAT-SAP is made with an antibody using a 22-amino acid peptide from porcine ChAT.

The targeted toxin has been shown in several papers to eliminate cholinergic neurons in the rat brain[3-6] (also see Cover Article) and is expected to cross-react with mouse, and many other species.



Representative sections of ChAT-immunostained tissues of N.Acc. from rats that (A and C) received an intra-accumbens micro-injection of Rabbit IgG-SAP (Cat. #IT-35; 250 ng; control), and (B and D) received an intra-accumbens micro-injection of Anti-ChAT-SAP (250 ng). Administration of Anti-ChAT-SAP reduced significantly the amount of cholinergic interneurons at the injection site while sparing adjacent areas. Scales A and B = 200 µm; C and D = 1 mm; ac: anterior commissure.
François LaPlante. Targeting Trends, 2013. 14(1): p. 1,6.

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