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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).

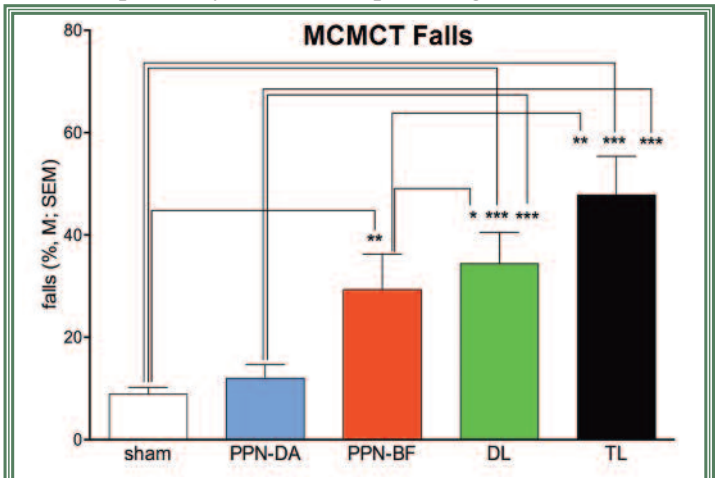

Fig. 1. Rats with PPN + BF cholinergic cell losses, with BF cholinergic and striatal dopamine lesions (DL), and with triple system lesions (TL) fell more than shams from the rotating rod (alternating directions).

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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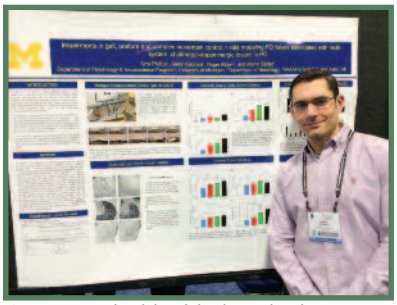
Inside this issue:

Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

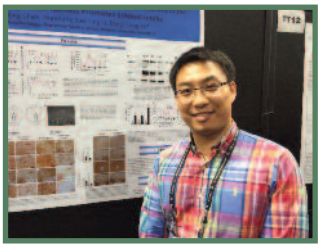
Society for Neuroscience Poster of the Year Award



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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Protein A quantification kit *(Cat. #RDB-06)*

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.

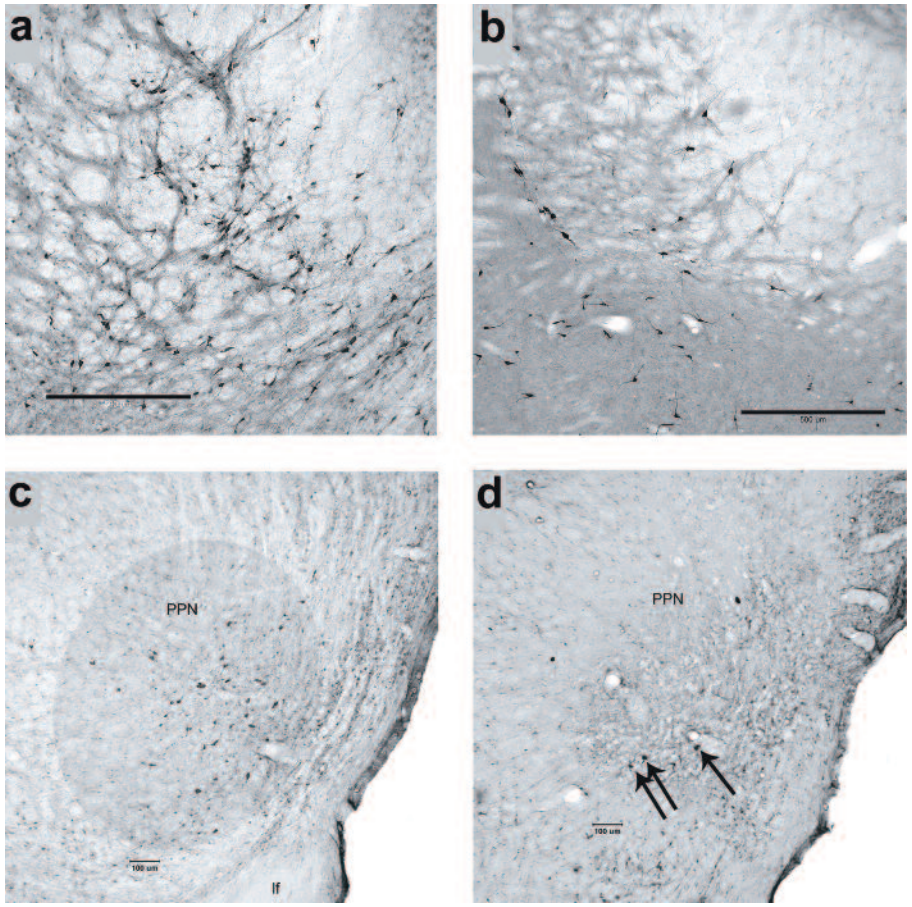

Fig. 2. (a) ChAT immunoreactivity on a coronal section of the BF from a sham-lesioned rat. (b) Infusions of 192-IgG saporin removed 70-90% of the cholinergic neurons in this region. (c) Cholinergic neurons in the intact PPN (shaded region; ChAT-immunoreactivity). d) Infusions of anti-ChAT-SAP lesioned >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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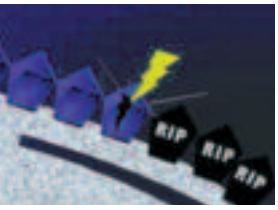
§ or anything recognized on the cell surface and internalized.

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