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Volume 15, Issue 1

Targeting Trends

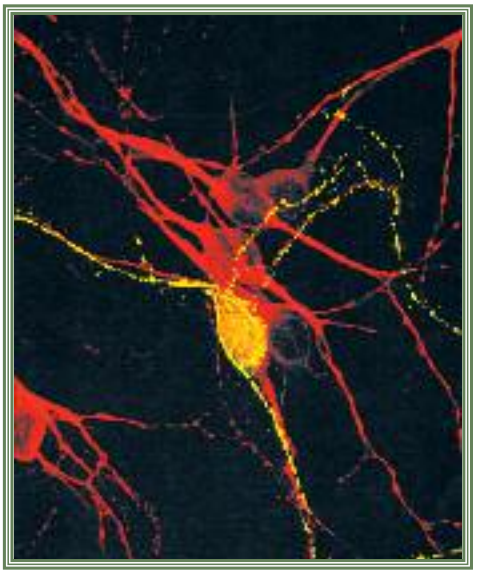
Reporting the latest news in Molecular Surgery

FDA Gives Green Light to Human Clinical Trials for Cancer Pain

Contributed by Douglas A. Lappi, Ph.D., President/Chief Scientific Officer, Advanced Targeting Systems, San Diego, CA

Substance P-Saporin (SP-SAP), ATS's patented conjugate being developed for cancer pain therapy, has attracted a considerable amount of attention recently. Back-to-back publications, a press release and editorial were featured in the November issue of the journal *Anesthesiology*.

The first article, by Wiese *et al*.,[1] described the FDA-required intrathecal SP-SAP safety study in the dog. The authors concluded that intrathecal 15-µg SP-SAP reduced dorsal, but not ventral, neurokinin 1 receptor-positive (NK1r, the receptor for substance P) neurons at the spinal level of delivery with minimal side effects, whereas 150-µg SP-SAP resulted in motor neuron toxicity. Immunohistochemistry using a new NK1r antibody was correlated with *in situ* hybridization, and showed equality of identification on NK1r-expressing neurons.



Yellow staining for saporin after internalization of SP-SAP by this single spinal cord neuron.
Photo Credit: S. Rogers, J. Ghilardi, P. Mantyh (University of Minnesota)

The second study was in dog cancer pain performed by Brown and Agnello[2] at the University of Pennsylvania that showed, as the title states, "Intrathecal substance P-saporin in the dog: efficacy in bone cancer pain." Companion (pet) dogs were randomized into a group that was going through normal pain management and a group that was treated with normal pain management, but received SP-SAP. Owners were kept in consult and the primary endpoint was measured as when the owners requested more pain pharmaceutical (dog activity, pain scores, and videography data were also collected). There was a significant difference between the two groups: more dogs in the control group that did not receive SP-SAP (74%) required unblinding and adjustment in analgesic protocol or euthanasia within 6 weeks of randomization than dogs that were treated with SP-SAP (24%; $P<0.001$).

The editorial comment by Hayashida[3] discusses aspects of the NK1r approach. Substance P has long been known to be important in normal pain transmission, but in the late 90's, its receptor in the spinal cord began to be implicated in pathological pain. Substance P antagonists did not provide relief in human trials, so, Hayashida asks, why then would elimination of NK1r–expressing spinal neurons work? It is suggested that blockade of only one type of receptor is not sufficient, because of all the other neurotransmitters of pain (CGRP and glutamate, for instance) that still function on the pathology-producing neurons. Of great importance is that removal of NK1r-positive neurons removes the pathology of chronic pain, but there are other cells functioning on the input of other transmitters that allow for continued perception of normal acute pain.

The Food and Drug Administration has now allowed the use of SP-SAP in clinical trial. The

(continued on page 6)

Inside this issue:

Targeting Topics
Scientific References 3

Targeting Talk
Questions & Answers 5

Targeting Tools
Featured Products 7

Targeting Teaser
Word Quiz 8

Newsletter Highlights

- Tribute to Gangsta: a much-loved cat bids us adieu *(pics on pages 2-7)*
- Spring Brain Conference 25th Anniversary *(page 2)*
- Research tools to Investigate Itch *(page 7)*
- Targeting Teaser Win a Tote Bag! *(page 8)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Spring Brain Conference: 25th Anniversary
Sedona, Arizona March 19-22, 2014

Program at a Glance	
Wednesday	
6pm-6:30pm	Reception (food and beverages provided)
7pm-8:30pm	Introduction of Conference Speakers Opening Ceremony & Guest Speakers
Thursday & Friday	
7am-8am	Breakfast Buffet
8am-12:00pm	Plenary Sessions
12:00pm	Boxed Lunches to Go - available to pickup 11am at Conference Registration table
12pm-6pm	Free Time (golf, hike, tennis, spa, jeep tours, etc)
6pm-7pm	Happy Hour - Posters / Discussions
7:00-7:30pm	Dinner Buffet
7:30-8:30pm	Keynote Address
Saturday	
7am-8am	Breakfast Buffet
8am-12:00pm	Plenary Sessions
12:00pm-1:00pm	Lunch Buffet
View the Detailed Program Schedule	

The meeting starts Wednesday evening with a cocktail reception, introductions of conference speakers and poster presenters. There will be a surprise, very special, guest speaker and entertainment that will kick off a splendid three days of science, learning, conversation and fun, all in a beautiful location with golf, hiking, art galleries, and museums. The keynote speakers this year are impressive. On Thursday night, Baldomero Olivera, the discoverer of PriAlt (Ziconotide), the conotoxin approved for cancer pain, will discuss his fabulous stories of the myriad of toxins from animals that build gorgeous sea shells. On Friday night, Michael Merzenich (UCSF) will provide the Keynote Address. Dr. Merzenich was honored by election into the National Academy of Sciences for his research on brain plasticity.

On Thursday, Friday, and Saturday, the morning sessions begin at 8am and include the following highlights: Howard Eichenbaum will give a special talk on his fundamental work on Learning and Memory; Pain and the Brain (Frank Porreca, Session Leader, has invited Allan Basbaum and Rob Caudle to present the latest in the pain field); Somatosensory Issues with Mark A. Hoon, NIH as Session Leader (check out Dr. Hoon's recent publication in *Science* on itch and how it works). David Lyon, Jim Marshel, and Prakash Kara will discuss functional circuits in the visual cortex, and Patrick Mantyh and Doug Lappi will finish up the meeting with a presentation on "Relief of Skeletal and Cancer Pain."

Interspersed in the sessions will be poster presenters with short presentations and Q&A. If you have a poster you'd like to share (maybe a poster presented at SfN?), please check out the instructions online (http://springbrain.org/abstracts.html) and submit by February 15.

Targeting Teaser Solution

Solve this quarter's teaser at www.ATSbio.com/news/14q1_teaser.html

Congratulations to the puzzle solvers from last quarter. Each winner has received a tote bag featuring the 25th Annual Spring Brain Conference.

The solution to the puzzle was:

Jumbles: NEUROSCIENCE
MORPHINE
BACKFIRE
TRANSPORTER
CHLORIDE

What the scarecrow 'thought' he needed to become a neuroscientist.

Answer: IF I ONLY HAD... A BRAIN.

WINNERS: Glenn H. Kageyama, Cal Poly Pomona Univ* Kishore Kotta, UCSD * Clay Archer, UCSD * Shikha, City of Hope * Travis Kroeker, NY Stem Cell Fdtn * Chelsea Friedman, UC Berkeley * Tsung-Chang Sung, Salk Inst * Chia-Yun Tseng, Salk Inst * Peter Syapin, Texas Tech Univ HSC * Khalid Touzani, Brooklyn College of CUNY * Bertha Dominguez, Salk Inst * Val Fritz, Frostburg State Univ * Judene Bliss, Roswell Park Cancer Inst * Florian Sweeney, Salk Inst * Michael Conner, Children's Hosp Philadelphia * Kristen Phend, Univ North Carolina Chapel Hill * Yelena Dayn, Salk Inst

"A cat has absolute emotional honesty: human beings, for one reason or another, may hide their feelings, but a cat does not."
- Ernest Hemingway

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

BMP9 ameliorates amyloidosis and the cholinergic defect in a mouse model of Alzheimer's disease.

Burke RM, Norman TA, Haydar TF, Slack BE, Leeman SE, Blusztajn JK, Mellott TJ.

Proc Natl Acad Sci U S A 110(48):19567-19572, 2013.

During development bone morphogenetic protein 9 (BMP9) induces the cholinergic phenotype in the basal forebrain. The authors investigated the use of BMP9 as a treatment of basal forebrain cholinergic degeneration, such as is seen in Alzheimer's disease (AD). Transgenic mice displaying AD phenotypes and expressing GFP in cholinergic neurons received icv infusions of BMP9, and several cholinergic markers were assessed. Anti-p75NTR (Cat. #AB-N01) was used in immunoblotting at a 1:3000 dilution to measure p75 levels. The results demonstrate the protective and therapeutic activity of BMP9 on AD symptoms.

Modeling fall propensity in Parkinson's disease: deficits in the attentional control of complex movements in rats with cortical-cholinergic and striatal-dopaminergic deafferentation.

Kucinski A, Paolone G, Bradshaw M, Albin RL, Sarter M.

J Neurosci 33(42):16522-16539, 2013.

Parkinson's disease produces a range of symptoms, some of which are unresponsive to therapies such as levodopa. These nonmotor symptoms include cognitive impairments and deficiencies in gait and balance. Here the authors develop a system to assess fall propensity in rats and examine the interaction between loss of cortical cholinergic and striatal dopaminergic afferents. Rats received 160-ng injections of 192-IgG-SAP (Cat. #IT-01) into the nucleus basalis and substantia innominata of the basal forebrain. The results indicate that the dual lesions result in diminished striatal control of complex movement.

Intrathecal substance p-saporin in the dog: efficacy in bone cancer pain.

Brown DC, Agnello K.

Anesthesiology 119(5):1178-1185, 2013.

This work demonstrates the use of naturally occurring bone cancer in dogs as a model for pain therapy. Companion dogs with bone cancer received 20-60 µg intrathecal injections of SP-SAP (currently in human clinical trials, see cover article) depending on the size of the dog. Significantly more dogs in the control group required unblinding and adjustment of pain care than in the SP-SAP group, indicating the efficacy of SP-SAP in pain control. This study also demonstrates the validity of the dog model for testing analgesic protocols.

Intrathecal substance p-saporin in the dog: distribution, safety, and spinal neurokinin-1 receptor ablation.

Wiese AJ, Rathbun M, Butt MT, Malkmus SA, Richter PJ, Osborn KG, Xu Q, Veesart SL, Steinauer JJ, Higgins D, Lappi DA, Russell B, Yaksh TL.

Anesthesiology 119(5):1163-1177, 2013.

Here the authors investigate the safety parameters of SP-SAP on purpose-bred beagles (currently in human clinical trials, see cover article). The dogs received 1.5, 15, or 150 µg intrathecal injections of the conjugate. SP-SAP pharmacology and physiological effects were assessed by behavioral and functional observations, immunohistochemistry, ELISA, blood and urine analysis, histopathology, and *in situ* hybridization. The general conclusions include that neurokinin-1 receptor (NK1r) positive neuron loss is detectable as soon as 7 days after administration of SP-SAP, the neuron loss is permanent, toxicity is specific to NK1r-positive neurons, and, other than the 150 µg dose, NK1r neuron loss was restricted to the superficial dorsal horn.

Substance P-Saporin for Bone Cancer Pain in Dogs: Can Man's Best Friend Solve the Lost in Translation Problem in Analgesic Development?

Hayashida K.

Anesthesiology 119(5):999-1000, 2013.

This editorial describes the SP-SAP papers in this latest issue of *Anesthesiology* (See summaries above). The results of the paper are discussed, and the potential in using companion dogs for pain models is emphasized. While most pain models have been rodent-based, companion dogs provide models for chronic pain due to natural causes such as cancer and arthritis, along with frequent opportunity for behavioral assessments by the owner. Such assessments can be done without stress to the animal.

Concordance between *in vivo* and postmortem measurements of cholinergic denervation in rats using PET with [18F]FEOBV and choline acetyltransferase immunochemistry.

Parent MJ, Cyr M, Aliaga A, Kostikov A, Schirrmacher E, Soucy JP, Mechawar N, Rosa-Neto P, Bedard MA.

EJNMMI Res 3(1):70, 2013.

Positron emission tomography (PET) imaging agents have been developed for the quantitative evaluation of cholinergic systems *in vivo*, and in this work the authors examine the concordance between the *in vivo* use of PET and post-mortem analysis of cholinergic damage. Rats received unilateral 0.2-0.25 µg injections of 192-IgG-SAP (Cat. #IT-01) into the nucleus basalis magnocellularis. Animals were scanned using [18F]fluoroethoxybenzovesamicol, then sacrificed for cholineacetyltransferase immunohistochemistry. The results support the use of PET as an *in vivo* method for analyzing the loss of cholinergic neurons.

Hindbrain Catecholamine Neurons Control Rapid Switching of Metabolic Substrate Use during Glucoprivation in Male Rats.

Li AJ, Wang Q, Dinh TT, Wiater MF, Eskelsen AK, Ritter S.

Endocrinology 154(12):4570-4579, 2013.

Previous work has shown that corticosterone secretion in response to glucoprivation is at least in part controlled by hindbrain catecholamine neurons in the paraventricular nucleus of the hypothalamus (PVH). In this

"Of all God's creatures, there is only one that cannot be made slave of the lash. That one is the cat. If man could be crossed with the cat it would improve the man, but it would deteriorate the cat."
- Mark Twain

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

work the authors investigate the metabolic consequences of lesioning these neurons. Rats received bilateral 82-ng infusions of Anti-DBH-SAP (Cat. #IT-03) into the PVH. Saporin (Cat. #PR-01) was used as a control. Although lesioned animals had the same energy expenditure and locomotor activity as controls, they also had a higher respiratory exchange ratio, indicating a reduced ability to switch from carbohydrate to fat metabolism in response to glucoprivation.

Selective lesioning of nucleus incertus with corticotropin releasing factor-saporin conjugate.

Lee LC, Rajkumar R, Dawe GS.
Brain Res Epub2013.

In this work the authors used CRF-SAP (Cat. #IT-13) to eliminate CRF1 receptor-expressing cells from the nucleus incertus (NI). Rats received bilateral CRF-SAP injections of 21.5 to 86 ng into the NI. Blank-SAP (Cat. #IT-21) was used as a control. Lesioned animals displayed a significant loss of CRF1 receptor-expressing cells, along with a decrease in relaxin-3 and GAD65 expression.

Selective potentiation of (alpha4)3(beta2)2 nicotinic acetylcholine receptors augments amplitudes of prefrontal acetylcholine- and nicotine-evoked glutamatergic transients in rats.

Grupe M, Paolone G, Jensen AA, Sandager-Nielsen K, Sarter M, Grunnet M.
Biochem Pharmacol 86(10):1487-1496, 2013.

Nicotinic acetylcholine receptors (nAChR) are involved in a wide range of processes in the central nervous system, many having to do with higher cognitive functions. In order to better understand how these receptors mediate attentional performance, the authors investigated glutamate release under varying conditions. In one series of experiments rats received a 160-ng injection of 192-IgG-SAP (Cat. #IT-01) into the right medial prefrontal cortex. The resulting decrease in glutamate release after the cholinergic lesion adds to the data indicating that positive modulation of nAChR may help alleviate attentional impairments caused by some brain disorders.

"The smallest feline is a masterpiece."
- Leonardo da Vinci

Hindbrain noradrenergic input to the hypothalamic PVN mediates the activation of oxytocinergic neurons induced by the satiety factor oleoylethanolamide.

Romano A, Potes CS, Tempesta B, Cassano T, Cuomo V, Lutz T, Gaetani S.
Am J Physiol Endocrinol Metab 305(10):E1266-73, 2013.

Feeding behavior and energy balance are in part controlled by signals from the gut. Oleoylethanolamide (OEA) is an acylethanolamide that is thought to play a role in this network. Since peripheral administration of OEA has effects on the nucleus of the solitary tract (NTS) and paraventricular nucleus (PVN) the authors investigated the role of noradrenergic afferent input to these areas. Rats received bilateral 84-ng injections of Anti-DBH-SAP (Cat. #IT-03) into the PVN. Mouse IgG-SAP (Cat. #IT-18) was used as a control.

Oscillatory coupling within neonatal prefrontal-hippocampal networks is independent of selective removal of GABAergic neurons in the hippocampus.

Bitzenhofer SH, Hanganu-Opatz IL.
Neuropharmacology 77C:57-67, 2013.

During cognitive tasks neuronal networks are entrained by oscillatory electrical rhythms with different frequencies. It has been proposed that GABAergic neurons in the prefrontal-hippocampal networks control this processing. The authors administered 252 ng of Anti-vGAT-SAP (Cat. #IT-70) into the ventral hippocampus of rats to examine how the GABAergic neurons could be involved. Unconjugated anti-vGAT (Cat #AB-N44) was used as a control. Hippocampal sharp waves were impaired during neonatal development, but the data indicate that oscillatory coupling between the neonatal prefrontal cortex and hippocampus is not controlled by GABAergic hippocampal interneurons.

Effects of intrathecal SNC80, a delta receptor ligand, on nociceptive threshold and dorsal horn substance p release.

Kouchek M, Takasusuki T, Terashima T, Yaksh TL, Xu Q.
J Pharmacol Exp Ther 347(2):258-264, 2013.

In this work the authors utilized three different preclinical pain models to examine the effects of intrathecal administration of the DOR agonist SNC80. One strategy was to assess NK-1 receptor (NK1r) internalization using anti-NK1r (Cat. #AB-N33AP) in IHC at a 1:3000 dilution on cryosections. The data indicate that the transmitter release from small peptidergic afferents is an effect mediated by DOR in the spinal cord.

A1 noradrenergic neurons lesions reduce natriuresis and hypertensive responses to hypernatremia in rats.

da Silva EF, Freiria-Oliveira AH, Custodio CH, Ghedini PC, Bataus LA, Colombari E, de Castro CH, Colugnati DB, Rosa DA, Cravo SL, Pedrino GR.
PLoS One 8(9):e73187, 2013.

Using bilateral 63-ng injections of Anti-DBH-SAP (Cat. #IT-03) into two levels of the caudal ventrolateral medulla, the authors assessed several pressor responses to infusion of hypertonic saline. Saporin (Cat. #PR-01) was used as a control. The results suggest that medullary noradrenergic A1 neurons are involved in the regulation of some responses to acute changes in body fluid composition.

Lesion of the commissural nucleus of the solitary tract/A2 noradrenergic neurons facilitates the activation of angiotensinergic mechanisms in response to hemorrhage.

Freiria-Oliveira AH, Blanch GT, De Paula PM, Menani JV, Colombari DS.
Neuroscience 254:196-204, 2013.

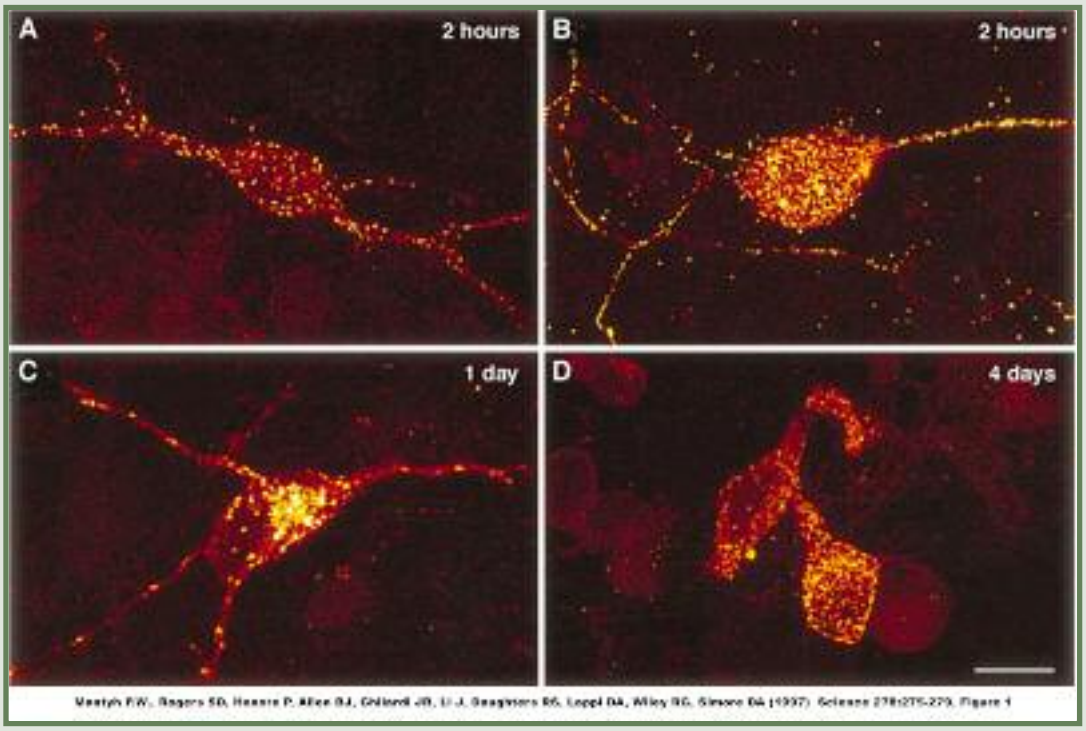
Previous work has generated conflicting data on the role of catecholaminergic A2 neurons in the nucleus of the solitary tract (NTS) in control of arterial pressure lability. The authors used Anti-DBH-SAP (Cat. #IT-03) to

(continued on page 6)

Targeting Talk: Product Questions

Q: How long does it take to see the cell death occurring from the use of targeted toxins using saporin? Is there a time course of hours or days?

A: The figure below illustrates the time course of cell death very effectively.



Internalization and cytotoxicity of SP-SAP in primary cultures of neonatal spinal cord neurons (11). Confocal image of neurons where the SPR immunofluorescence (A, C, D) appears red, areas of concentrated SPR immunofluorescence appear yellow. (A, C, and D) SPR immunofluorescence in neurons 2 hours, 1 day, and 4 days, respectively, after treatment with SP-SAP. (B) Confocal image showing SAP immunofluorescence (yellow) 2 hours after SP-SAP treatment.

These images were projected from 14 optical sections acquired at 0.8-mm intervals with a 603 lens. Bar, 25 mm.

Amer Assoc Cancer Res
April 5-9, 2014
San Diego, CA
Booth #1645

Upcoming Events

Experimental Biology
April 26-30, 2014
San Diego, CA
Booth #1342

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"There are two means of refuge from the miseries of life: music and cats."
- Albert Schweitzer

SP-SAP Cancer Pain Treatment in Human Clinical Trials

(continued from page 1)

patient population will be terminal cancer patients in pain that is refractory to opioids, a needy population indeed. The clinical trial will occur at the University of Texas Southwestern Medical Center in Dallas, under the direction of Dr. Carl Noe of the Department of Anesthesiology and Pain Management and sponsored by Dr. Arthur Frankel of UTSW's Simmons Comprehensive Cancer Center. Dr. Frankel is a leading expert in the use of targeted toxins in humans. As Dr. Hayashida states: "We are looking forward to results of this clinical study."

References

1. Wiese AJ, Rathbun M, Butt MT et al. Intrathecal substance P-saporin in the dog: distribution, safety, and spinal neurokinin-1 receptor ablation. *Anesthesiology*. 2013;119:1163-1177
2. Brown DC, Agnello K. Intrathecal substance P-saporin in the dog: efficacy in bone cancer pain. *Anesthesiology*. 2013;119:1178-1185
3. Hayashida K. Substance P-saporin for bone cancer pain in dogs: can man's best friend solve the lost in translation problem in analgesic development? *Anesthesiology*. 2013;119:999-1000

Targeting Topics: Recent Scientific References

(continued from page 4)

lesion these neurons in a hypotensive hemorrhage model. Rats received two injections of 12.6 ng into the commissural NTS. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The lesioned animals quickly recovered from hypotension, but were impaired by the icv administration of losartan.

Impaired hippocampal acetylcholine release parallels spatial memory deficits in Tg2576 mice subjected to basal forebrain cholinergic degeneration.

Laursen B, Mork A, Plath N, Kristiansen U, Frank Bastlund J.

Brain Res Epub2013.

The Tg2576 mouse strain provides a limited model for Alzheimer's disease because they do not display degeneration of cholinergic neurons in the basal forebrain – the other main hallmark of Alzheimer's disease in humans. Using 0.9 µg icv injections of mu p75-SAP (Cat. #IT-16) the authors evaluated mice that had both Aβ deposition and cholinergic depletion. The data show that these mice display cognitive decline and compromised cholinergic levels, creating a viable model for Alzheimer's disease.

"Cats are a mysterious kind of folk. There is more passing in their minds than we are aware of."

- Sir Walter Scott

Characterization of cultured multipotent zebrafish neural crest cells.

Kinikoglu B, Kong Y, Liao EC.

Exp Biol Med (Maywood) Epub2013.

This work details the isolation of neural crest cells (NCCs) from transgenic zebrafish embryos expressing GFP and flow cytometry; the authors analyzed lineage markers and differentiation of the NCCs. Anti-mu p75 (Cat. #AB-N01AP) was used in immunocytochemistry at a 1:20 dilution on fixed cells.

Selective immunotoxic lesions of basal forebrain cholinergic cells: effects on learning and memory in rats.

Baxter MG, Bucci DJ, Gorman LK, Wiley RG, Gallagher M.

Behav Neurosci 127(5):619-627, 2013.

In this reprint of a 1995 article, 192-IgG-SAP (Cat. #IT-01) was used to separate the depletion of cortical cholineacetyltransferase and behavioral impairment – which had previously been linked by research using less specific lesioning methods. Since the original 1995 publication, hundreds of papers have been published using a variety of lesioning techniques and a wide range of ATS products.

Immunoablation of cells expressing the NG2 chondroitin sulphate proteoglycan.

Leoni G, Rattray M, Fulton D, Rivera A, Butt AM.

J Anat Epub2013.

In this work the authors use an antibody against the NG2-glia marker chondroitin sulphate proteoglycan (CSPG) along with Mab-ZAP (Cat. #IT-04) on cell lines and brain slices to eliminate cells expressing CSPG. The results demonstrate selective and effective killing, providing a method to study the function of these cells.

Deletion of naive T cells recognizing the minor histocompatibility antigen HY with toxin-coupled peptide-MHC class I tetramers inhibits cognate CTL responses and alters immunodominance.

Hess SM, Young EF, Miller KR, Vincent BG, Buntzman AS, Collins EJ, Frelinger JA, Hess PR.

Transpl Immunol 29(1-4):138-145, 2013.

The authors utilized biotinylated peptide-MHC class I tetramers with Streptavidin-ZAP (Cat. #IT-27) to selectively delete a specific population of alloreactive T cells in mice. Animals received iv 33-pmol injections of the toxic tetramer, and the data indicate that these toxic tetramers can prevent the induction of donor-specific responses that result in organ rejection.

Targeting Tools: Investigating the Itch Pathway

Bombesin-SAP (Cat. #IT-40)

Bombesin is a 14-amino-acid peptide found in frog-skin. The human equivalent, gastrin releasing peptide (GRP) has been detected in itch pathways and plays a role in eating behaviors. GRP regulates numerous functions of the gastrointestinal and central nervous systems, including release of gastrointestinal hormones, smooth muscle cell contraction, and epithelial cell proliferation.

Bombesin-SAP specifically targets GRP receptor-positive cells (Figure 1).[1] All other cells are left untouched. Elimination of cells expressing GPR receptor is useful in studying the role of bombesin in itch and eating behaviors.

Effective Tool: Elimination of cells expressing GRP receptor is useful in studying the role of bombesin in itch and eating behaviors.

1. Chen ZF, Sun YG, Zhao ZQ, Meng XL, Yin J, Liu XY(2009) Ablation of GRPR+ Neurons in the Spinal Cord by Bombesin-Saporin Knocks Out Itch Sensation in Mice Without Affecting Pain Circuit. Targeting Trends 10(4):1, 6.

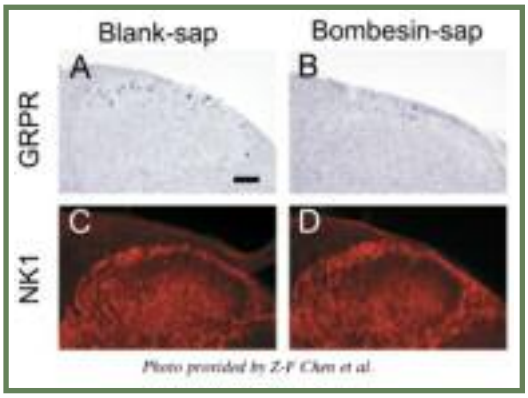


Figure 1. Selective ablation of GRPR+ neurons in the spinal cord. (A-B) GRPR expression detected by *in situ* hybridization significantly reduced in lamina I of mice. (C-D) NK1 receptor expression detected by immunocytochemistry in the dorsal horn was similar between the two groups. Scale bar: 100 μm.

Nppb-SAP (Cat. #IT-69)

Itch is triggered by somatosensory neurons expressing the ion channel TRPV1 (transient receptor potential cation channel subfamily V member 1). Neuropeptide natriuretic polypeptide b (Nppb) is expressed in a subset of TRPV1 neurons and research has found that Nppb lesioned mice selectively lose almost all behavioral responses to itch-inducing agents. Itch responses are blocked by toxin-mediated ablation of Nppb-receptor–expressing cells, but a second neuropeptide, gastrin-releasing peptide (Bombesin), still induces strong responses in the toxin-treated animals, an apparent downstream effect. **Nppb-SAP eliminates cells expressing neuropeptide natriuretic polypeptide b (Nppb or BNP) receptor.**

Mishra SK, Hoon MA. (2013) The cells and circuitry for itch responses in mice. Science 340(6135):968-971. (Targeting Trends 13q3)

NMB-SAP (Cat. #IT-70)

Neuromedin B (NMB) and GRP are two members of the mammalian bombesin family of peptides. These two peptides activate structurally similar but pharmacologically distinct G-protein-coupled receptors. NMB is expressed in a subset of sensory neurons that co-label with calcitonin gene-related peptide and TRPV1, suggestive of a role for NMB in nociception. **NMB-SAP removes neurons expressing the NMB receptor.** In the periphery NMB and GRP have a wide variety of actions including smooth muscle contraction and exocrine and endocrine functions. In the CNS these peptides regulate food intake and body temperature, as well as stress behavioral responses. Additionally, immunolocalization studies showed that NMB protein is present in the dorsal horn of the spinal cord and expression was also seen in sensory neurons.

Mishra SK, Holzman S, Hoon MA. (2012) A nociceptive signaling role for neuromedin B. J Neurosci 20(32):8686-8695. (Targeting Trends 12q4)

ON A CAT AGING
by Alexander Gray

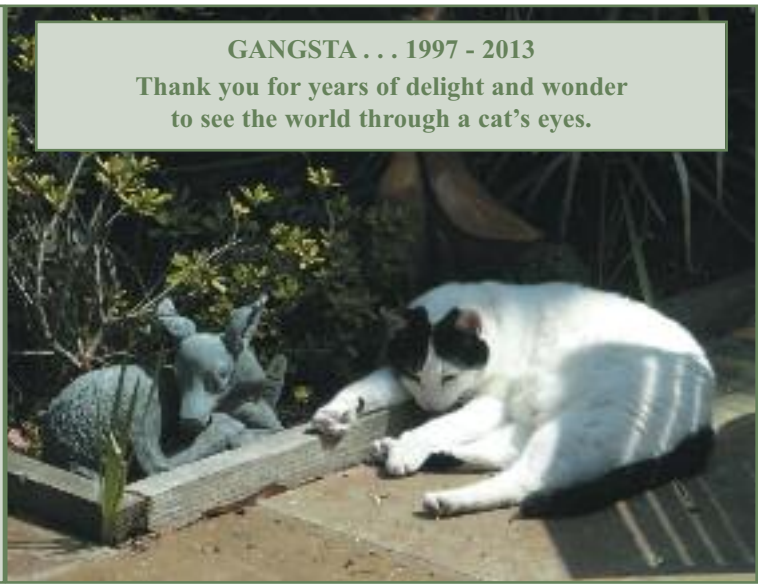
He blinks upon the hearth-rug,
and yawns in deep content,
accepting all the comforts
that Providence has sent.

Louder he purrs, and louder,
in one glad hymn of praise
for all the night's adventures,
for quiet, restful days.

Life will go on for ever,
with all that cat can wish:
warmth and the glad procession
of fish and milk and fish.

Only-the thought disturbs him-
he's noticed once or twice,
the times are somehow breeding
a nimbler race of mice.

GANGSTA . . . 1997 - 2013
Thank you for years of delight and wonder to see the world through a cat's eyes.



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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 the cover story**, one letter to each block, to solve the puzzle.

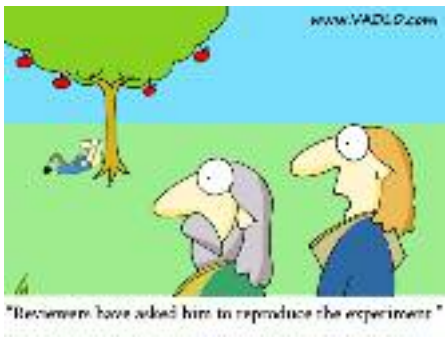
TRACEMAPCHULIA

CHEATLANTIR

WROBN

GLOOPHYAT

PRIDEYAVOGH



What the scientist said about studying gravity.

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

ANSWER:
JUST DON'T LET IT . . . ◯◯◯ ◯◯◯ ◯◯◯◯ !

WIN!

SOLVE the puzzle online with the correct solution by March 31, 2014.

WIN a large, reusable flat-bottom tote bag featuring the 25th Annual Spring Brain Conference!

www.atsbio.com/news/14q1_teaser.html

Apr-May-Jun 2014
Volume 15, Issue 2

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Targeting Topics
Summary of Scientific References 3

Targeting Talk
Questions & Answers 5

Targeting Tools
SfN Poster Winners 7

Targeting Teaser
Word Quiz 8

Newsletter Highlights

- 20 Years of Special People *(page 2)*
- Top 20 Teaser Winners *(page 2)*
- 20 Most-Cited References *(page 3)*
- Top Q&A *(page 5)*
- Targeting Teaser
  Win a 20th Anniversary Tote Bag! *(page 8)*

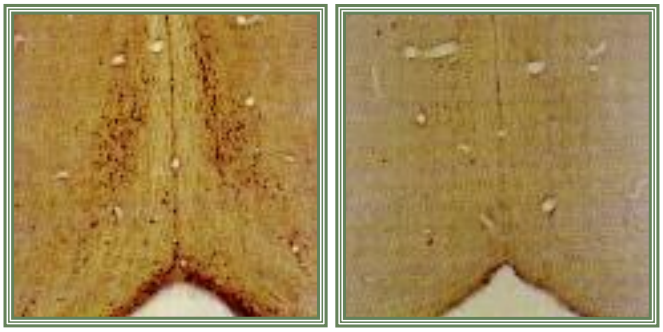
Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Celebrating Twenty Years of Science

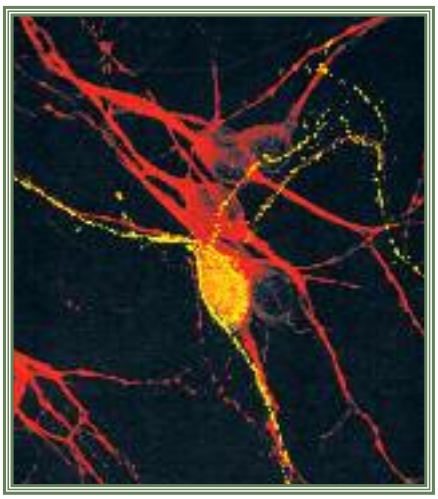
Advanced Targeting Systems (founded 22 Apr 1994)

Advanced Targeting Systems' first product, 192-IgG-SAP, answered a long-sought request from behavioral neuroscientists: a neurotoxin for the cholinergic neurons of the basal forebrain. Over the years it has become the classic in the field and has changed the way that we think about those neurons and their role in learning and memory.



CONTROL
$p75^{NTR}$-positive neurons of rat cholinergic forebrain.
Photo Credit:
C. Wrenn, R.G. Wiley

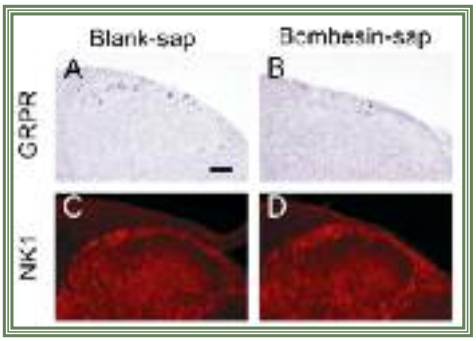
TREATED
icv injection of 192-IgG-SAP. >95% elimination of target neurons



Yellow staining for saporin after internalization of SP-SAP by this single spinal cord neuron.
Photo Credit: S. Rogers, J. Ghilardi, P. Mantyh

Substance P-saporin (SP-SAP) rapidly became an item of interest due to the impact on chronic pain models. As one renowned Neuroscientist stated: "No one expected these results," and they have been replicated many times over. Aside from that, SP-SAP opened new ways to analyze that jungle of fibers in the spinal cord and presaged a series of products that are dissecting and establishing function of neurons in the spinal cord.

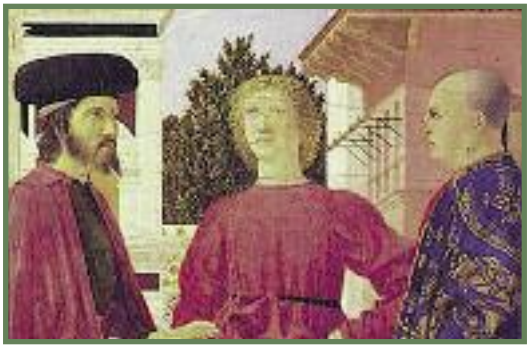
Bombesin-SAP is gastrin-releasing peptide (made from the frog skin version discovered by Esparmer and co-workers), attached to saporin. This targetd toxin made a splash by demonstrating that the urge to itch could be stopped by removing GRP receptor-positive neurons in the spinal cord. This led to renewed interests and approaches in the itch field and the relationship between pain and itch. A new series of toxins sprang forth to foster the discussion.



Selective ablation of GRPR+ neurons in the spinal cord. (A-B) GRPR expression detected by *in situ* hybridization.
(C-D) NK1 receptor expression detected by immunocytochemistry in the dorsal horn.
Scale bar: 100 μm.
Photo Credit: Z.F. Chen et al.

Celebrating Twenty Years of People Who Make ATS Special

Founder, President, and Chief Scientific Officer, Doug Lappi (20 yr)



The 3 Guys of Piero della Francesca

Founder, Vice President and *Targeting Trends* Editor, Denise Higgins (20 yr)

Christmas Lunch 2013: L to R are Brian Russell, Christian Nguyen (Lab Intern), Leonardo Ancheta, and Patrick Shramm (new Product Manager).

The 3 Guys of ATS: Product Managers Matt Kohls (15 yr), Brian Russell (13 yr), and Leonardo Ancheta (10 yr)

Above: Kristen Hartman (Website/Database Manager) and Below: Tom Cobb (Administration Manager)

Targeting Teaser Solution

The solution to the puzzle was:

Jumbles: PHARMACEUTICAL
INTRATHECAL
BROWN
PATHOLOGY
VIDEOGRAPHY

What the scientist said about studying gravity..

Answer: Just don't let it... GET YOU DOWN.

Solve this quarter's teaser at www.ATSbio.com/news/14q2_teaser.html

Congratulations to the puzzle solvers from last quarter. Each winner has received a tote bag featuring the 25th Annual SBC.

LAST QUARTER'S WINNERS: Glenn H. Kageyama, Cal Poly Pomona Univ * Kris Preddy, Lakeside, CA * Naroon Sakool, Univ Cal San Diego, La Jolla, CA * Mitchell Tse, Baxter Healthcare, Hayward, CA * Bill Henry, Rhode Island Hospital, Providence, RI * Laura Vitale, Celldex Therapeutics, Hampton, NJ * Elizabeth Lake, Fresno Pacific Univ, Fresno, CA * Mohammed Aziz, King Abdulla Intl Med Res Ctr, Riyadh, Saudi Arabia * Rheem D. Medh, Cal State Univ, Northridge, CA * Elizabeth Graham, SK Life Science, Fair Lawn, NJ * Volker Haring, CSIRO CAFHS, Victoria, Australia * MD Mamunur Rashid, SUNY Upstate Med Univ, Syracuse, NY * Patrick Stockwell, MCRF, Marshfield, WI * Herbert Geller, NHLBI, Bethesda, MD * Nuzhat Ahsan, National Inst Immunology, New Delhi, India * Debbie Livingston, Rancho Santa Margarita, CA

TOP TWENTY ALL-TIME WINNERS: Seto Chice (31x) * Robert Speth (31x) * Glenn H. Kageyama (18x) * Kristen Phend (9x) Joseph Menonna (8x) * Ruth Stornetta (8x) * Thea Marlinga (7x) * Bruce Pappas (7x) * April Price (6x) * Kim Van Vliet (6x) * Barbara Attadi (5x) * Shikha Gaur (5x) * Roger Guillemin (4x) * Shawn McClelland (4x) * Valery Nelson (4x) * Douglas J. Taatjes (4x) * Khalid Touzani (4x) * Vivian Yip (4x) * Lynn Young (4x) * Clay Archer (3x) * Judene Bliss (3x) * Angela Finney (3x) * Christopher Flores (3x) * Valerie Fritz (3x) * Bryan Hudson (3x)

Targeting Topics: Top 20 Most-Cited References

Kilgard MP, Merzenich MM (1998) Cortical map reorganization enabled by nucleus basalis activity. *Science* 279:1714-1718.
192-IgG-SAP (Cat. #IT-01)
888 Citations

Lu J, Sherman D, Devor M, Saper CB (2006) A putative flip-flop switch for control of REM sleep. *Nature* 441(1):589-594.
Orexin-SAP *494 Citations*

Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, Daughters RS, Lappi DA, Wiley RG, Simone DA (1997) Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. *Science* 278:275-279.
SP-SAP (see SSP-SAP, Cat. #IT-11)
486 Citations

Chen J, Zhou Y, Mueller-Steiner S, Chen LF, Kwon H, Yi S, Mucke L, Gan L (2005) SIRT1 protects against microglia-dependent amyloid-beta toxicity through inhibiting NF-kappaB signaling. *J Biol Chem* 280(48):40364-40374.
Acetylated LDL-SAP (Cat. #IT-08)
402 Citations

Berger-Sweeney J, Heckers S, Mesulam M-M, Wiley RG, Lappi DA, Sharma M (1994) Differential effects of spatial navigation of immunotoxin-induced cholinergic lesions of the medial septal area and nucleus basalis magnocellularis. *J Neurosci* 14:4507-4519.
192-IgG-SAP (Cat. #IT-01) *377 Citations*

Heckers S, Ohtake T, Wiley R, Lappi DA, Geula C, Mesulam M-M (1994) Complete and selective cholinergic denervation of rat neocortex and hippocampus but not amygdala by an immunotoxin against the p75 NGF receptor. *J Neurosci* 14:1271-1289.
192-IgG-SAP (Cat. #IT-01) *364 Citations*

Gray PA, Janczewski WA, Mellen N, McCrimmon DR, Feldman JL (2001) Normal breathing requires preBotzinger complex neurokinin-1 receptor-expressing neurons. *Nat Neurosci* 4(9):927-930.
SP-SAP (see SSP-SAP, Cat. #IT-11)
357 Citations

McGaughy J, Kaiser T, Sarter M (1996) Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. *Behav Neurosci* 110:247-265.
192-IgG-SAP (Cat. #IT-01) *339 Citations*

Nichols ML, Allen BJ, Rogers SD, Ghilardi JR, Honore P, Luger NM, Finke MP, Li J, Lappi DA, Simone DA, Mantyh PW (1999) Transmission of chronic nociception by spinal neurons expressing the substance P receptor. Science 286:1558-1561.
SP-SAP (see SSP-SAP, Cat. #IT-11)
335 Citations

Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH (2002) Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. *Nat Neurosci* 5 (12):1319-1326.
SP-SAP (see SSP-SAP, Cat. #IT-11)
327 Citations

Lee MG, Chrobak JJ, Sik A, Wiley RG, Buzsaki G (1994) Hippocampal theta activity following selective lesion of the septal cholinergic system. *Neuroscience* 4:1033-1047.
192-IgG-SAP (Cat. #IT-01) *325 Citations*

Wiley RG, Oeltmann TN, Lappi DA (1991) Immunolesioning: selective destruction of neurons using immunotoxin to rat NGF receptor. *Brain Res* 562:149-153.
192-IgG-SAP (Cat. #IT-01) *321 Citations*

Baxter MG, Bucci DJ, Gorman LK, Wiley RG, Gallagher M (1995) Selective immunotoxic lesions of basal forebrain cholinergic cells: effects on learning and memory in rats. *Behav Neurosci* 109:714-722.
192-IgG-SAP (Cat. #IT-01) *289 Citations*

Torres EM, Perry A, Blokland A, Wilkinson LS, Wiley RG, Lappi DA, Dunnett SB (1994) Behavioral, histochemical and biochemical consequences of selective immunolesions in discrete regions of the basal forebrain cholinergic system. *Neuroscience* 63:95-122.
192-IgG-SAP (Cat. #IT-01) *284 Citations*

Giulian D, Haverkamp LJ, Yu JH, Karshin W, Tom D, Li J, Kirkpatrick J, Kuo YM, Roher AE (1996) Specific domains of ß-amyloid from Alzheimer plaque elicit neuron killing in human microglia. *J Neurosci* 16:6021-6037.
Acetylated LDL-SAP (Cat. #IT-08)
273 Citations

Chiba AA, Bucci DJ, Holland PC, Gallagher M (1995) Basal forebrain cholinergic lesions disrupt increments but not decrements in conditioned stimulus processing. *J Neurosci* 15:7315-7322.
192-IgG-SAP (Cat. #IT-01) *236 Citations*

Burgess SE, Gardell LR, Ossipov MH, Malan Jr TP, Vanderah TW, Lai J, Porreca F (2002) Time dependent descending facilitation from the rostral ventromedial medulla maintains, but does not initiate, neuropathic pain. *J Neurosci* 22(12):5129-5136.
Dermorphin-SAP (Cat. #IT-12)
230 Citations

Truitt WA, Coolen LM (2002) Identification of a potential ejaculation generator in the spinal cord. *Science* 297(5586):1566-1569.
SSP-SAP (Cat. #IT-11) *227 Citations*

Wenk GL, Stoehr JD, Quintana G, Mobley S, Wiley RG (1994) Behavioral, biochemical, histological, and electrophysiological effects of 192 IgG-saporin injections into the basal forebrain of rats. *J Neurosci* 14:5986-5995.
192-IgG-SAP (Cat. #IT-01) *219 Citations*

Vnek N, Kromer LF, Wiley RG, Rothblat LA (1996) The basal forebrain cholinergic system and object memory in the rat. *Brain Res* 710:265-270.
192-IgG-SAP (Cat. #IT-01) *208 Citations*

NOTE: In order to make space for the 20th Anniversary special additions, this quarter's references have abbreviated summaries beginning on Page 4.

Please visit

www.ATSbio.com/news/14q2_news.html

to see the complete summaries.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Habenular kisspeptin modulates fear in the zebrafish.

Ogawa S, Nathan FM, Parhar IS.
Proc Natl Acad Sci U S A 111(10):3841-3846, 2014.

Zebrafish express kiss1 mRNA which is a conserved ortholog of the mammalian KISSI/KissI making zebrafish a viable model for investigating the role of kisspeptin in various brain systems. Animals received 1 µg of the custom conjugate kiss-SAP via an intracranial injection. Blank-SAP (Cat. #IT-21) was used as a control.

Selective actions of novel allosteric modulators reveal functional heteromers of metabotropic glutamate receptors in the CNS.

Yin S, Noetzel MJ, Johnson KA, Zamorano R, Jalan-Sakrikar N, Gregory KJ, Conn PJ, Niswender CM.
J Neurosci 34(1):79-94, 2014.

Using a variety of methods, the authors show that mGlu2 and mGlu4 form a hetero-complex in both rat and mouse tissues. An anti-mGluR2 (Cat. #AB-N32) was used in coimmunoprecipitation.

Medial septum-diagonal band of Broca (MSDB) GABAergic regulation of hippocampal acetylcholine efflux is dependent on cognitive demands.

Roland JJ, Stewart AL, Janke KL, Gielow MR, Kostek JA, Savage LM, Servatius RJ, Pang KC.
J Neurosci 34(2):506-514, 2014.

In order to better understand the relationship between these two neuronal populations the authors administered 552.5 ng of GAT-1-SAP (Cat. #IT-32) to the MSDB of rats in several injections.

Cholinergic contributions to supramodal attentional processes in rats.

Ljubojevic V, Luu P, De Rosa E.
J Neurosci 34(6):2264-2275, 2014.

The authors administered bilateral 40 ng injections of 192-IgG-SAP (Cat. #IT-01) to the nucleus basalis magnocellularis of rats.

Don't see your publication here? Send us a PDF at ats@ATSbio.com and we'll review it in the next issue of *Targeting Trends*.

Saporin Conjugated Monoclonal Antibody to the Transcobalamin Receptor TCblR/320 Is Effective in Targeting and Destroying Cancer Cells.

Quadros EV, Nakayama Y, Sequeira JM.
J Cancer Ther 4(6):1074-1081, 2013.

The authors used a custom conjugate of antibodies generated against the TCb1R and saporin to eliminate cancer cell lines in culture, applying the conjugate to cells in a dosing range of 0.156-5 nM, 2.5 nM to have the optimal effect.

Neutralization of Plasmodium falciparum Merozoites by Antibodies against PfRH5.

Douglas AD, Williams AR, Knuepfer E, Illingworth JJ, Furze JM, Crosnier C, Choudhary P, Bustamante LY, Zakutansky SE, Awuah DK, Alanine DG, Theron M, Worth A, Shimkets R, Rayner JC, Holder AA, Wright GJ, Draper SJ.
J Immunol 192(1):245-258, 2014.

PfRH5 binds basigin on erythrocytes, and through the use of anti-basigin (Cat. #AB-42), among other antibodies, the authors better characterized aspects of this binding that may be useful in preventing malaria.

The novel EpCAM-targeting monoclonal antibody 3-17I linked to saporin is highly cytotoxic after photochemical internalization in breast, pancreas and colon cancer cell lines.

Lund K, Bostad M, Skarpen E, Braunagel M, Krauss S, Duncan A, Hogset A, Selbo P.
MAbs 6(3)2014.

The authors used a biotinylated antibody that binds EpCAM combined with streptavidin-ZAP (Cat. #IT-27) to cause specific cytotoxicity on different cancer cell lines.

Depletion of alloreactive T cells by anti-CD137-saporin immunotoxin.

Lee SC, Seo KW, Kim HJ, Kang SW, Choi HJ, Kim A, Kwon BS, Cho HR, Kwon B.
Cell Transplant Epub2014.

The authors used a custom conjugate of anti-mouse CD137 and saporin to eliminate alloreactive T cells during T cell donor transfer in mice. Rat IgG-SAP (Cat. #IT-35) was used as a control.

Neuroprotective effects of donepezil against cholinergic depletion.

Cutuli D, De Bartolo P, Caporali P, Tartaglione AM, Oddi D, D'Amato FR, Nobili A, D'Amelio M, Petrosini L.
Alzheimers Res Ther 5(5):50, 2013.

Here the authors pre-treated rats with the aceytlcholinesterase inhibitor donepezil before administering 0.5 µg of 192-IgG-SAP (Cat. #IT-01) into each side of the medial septum.

Gabapentin increases extracellular glutamatergic level in the locus coeruleus via astroglial glutamate transporter-dependent mechanisms.

Suto T, Severino AL, Eisenach JC, Hayashida KI.
Neuropharmacology 81C:95-100, 2014.

Rats received a 0.25-µg injection of anti-DBH-SAP (Cat. #IT-03) into the locus coeruleus. Mouse IgG-SAP (Cat. #IT-18) was used as a control.

Stimulation of feeding by three different glucose-sensing mechanisms requires hindbrain catecholamine neurons.

Li AJ, Wang Q, Dinh TT, Powers BR, Ritter S.
Am J Physiol Regul Integr Comp Physiol 306(4):R257-64, 2014.

The authors administered 82 ng of anti-DBH-SAP (Cat. #IT-03) into the paraventricular nucleus as bilateral injections. Saporin (Cat. #PR-01) was used as a control.

Ablating Spinal NK1-Bearing Neurons Eliminates the Development of Pain and Reduces Spinal Neuronal Hyperexcitability and Inflammation From Mechanical Joint Injury in the Rat.

Weisshaar CL, Winkelstein BA.
J Pain Epub2014.

The authors examined the role of NK1r-expressing spinal cells in this pathway. Rats received 100 ng SSP-SAP (Cat. #IT-11) via

(continued on page 5)

Targeting Talk: Product Q&A

Q: Your recent issue of Targeting Trends stated that it was unlikely that saporin compounds or constituents would be excreted in urine or feces. However, you acknowledge that experimental data is lacking. Have there been any tests of animal urine or feces for saporin content? My animal care staff are concerned.

A: One of the reasons that no studies have been done on excretion of saporin is that there isn't much on the theoretical side to cause concern. The primary issue is that the quantity used in mice (and even rabbits) is so small that when looked at in human terms (i.e., an animal 10 to 100-times larger), the dosage becomes insignificant. The LD_{50} for saporin in mice is 4-8 mg/kg; that would translate in humans to more than you'll ever use! The immunotoxins, which contain only about 20% saporin by weight, really do not contain all that much saporin.

Looking at it another way, you need a concentration of about 100 nM to see even a vague hint of toxicity of saporin to cells. In human blood, that would correspond to 24 mg injected systemically into a person. It would be really expensive for anyone to get close to that number.

As far as urine and feces goes, the same calculations are appropriate, but there will be considerable degradation - the protein content in urine and feces is quite low and the probability is that you will be dealing with only saporin. Remember saporin is a plant protein that is related to proteins in foods that we eat (cucumbers, for example).

Reference

Stirpe F, Derenzini M, Barbieri L, Farabegoli F, Brown AN, Knowles PP, Thorpe PE (1987) Hepatotoxicity of immunotoxins made with saporin, a ribosome-inactivating protein from Saponaria officinalis. *Virchows Arch [B]* 53:259-271.

Q: Are there any studies which indicate what doses of saporin (by itself or compounded with an antibody) would be hazardous if ingested or injected (i.e. systemic dose level resulting in death or organ dysfunction).

A: When there is an antibody that does recognize a human epitope (the human p75-saporin immunotoxin that is used in rabbits, for example), at about 1 pM one sees the slightest bit of toxicity to cells. That translates, if injected by error into a human blood supply, to about 170 micrograms. That also is a gigantic dose. I am using very conservative numbers here, and the bottom line is that you cannot accidentally reach such dangerous levels under normal handling situations.

Having said all this, we still recommend that our customers take excellent care of themselves and we state clearly that precautions should be taken by people handling these materials, just as they should use precautions with all laboratory chemicals. Please refer to the data sheets provided with our products for safety instructions.

Upcoming Events

FENS
July 5-9, 2014
Milan, Italy
Booth #25

Society for Neuroscience
November 15-19, 2014
Washington, DC
Booth #1019

(continued from page 4)

lumbar puncture. Blank-SAP (Cat. #IT-21) was used as a control.

The p75 receptor is associated with inflammatory thermal hypersensitivity.

Watanabe T, Ito T, Inoue G, Ohtori S, Kitajo K, Doya H, Takahashi K, Yamashita T.
J Neurosci Res 86(16):3566-3574, 2008.

The authors investigated the role that the p75 neurotrophin receptor might play in NGF-induced hyperalgesia. Mice received 10 µl of polyclonal anti-p75 (Cat. #AB-N01) into the plantar surface of the paw.

IB4(+) and TRPV1(+) sensory neurons mediate pain but not proliferation in a mouse model of squamous cell carcinoma.

Ye Y, Bae S, Viet CT, Troob S, Bernabe D, Schmidt BL.
Behav Brain Funct 10(1):5, 2014.

Mice received 2.5 µg intrathecal injections of IB4-SAP (Cat. #IT-10). Saporin (Cat. #PR-01) was used as a control.

Phox2b-expressing retrotrapezoid neurons and the integration of central and peripheral chemosensory control of breathing in conscious rats.

Takakura AC, Barna BF, Cruz JC, Colombari E, Moreira TS.
Exp Physiol 99(3):571-585, 2014.

The authors used bilateral lesions of the RTN with 0.3 to 1.2 ng total of SSP-SAP (Cat. #IT-11) to eliminate neurokinin-1 receptor-expressing neurons.

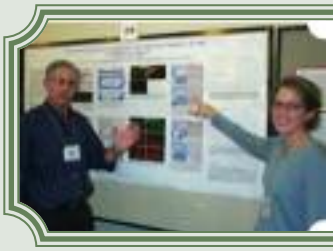
Expression of Different Neurokinin-1 Receptor (NK1R) Isoforms in Glioblastoma Multiforme: Potential Implications for Targeted Therapy.

Cordier D, Sailer M, Gerber A, Kluba C, Bauman A, Hutter G, Mindt TL, Mariani L.
Cancer Biother Radiopharm Epub2014.

SSP-SAP (Cat. #IT-11) was used at a concentration of 1 nM in cytotoxicity assays on several different glioma cell lines.

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

Targeting Stars: SfN Poster Winners



2000

Robert Sloviter and **Jennifer Martin** used SSP-SAP (Cat# IT-11)

2001

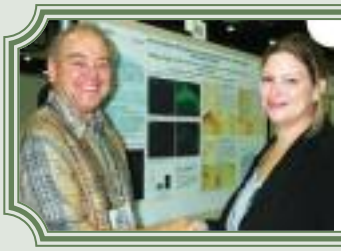
Mary Ann Greco used Orexin-SAP

2002

Lique Coolen and **William Truitt** used SSP-SAP (Cat# IT-11)

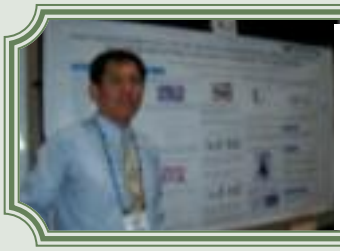
2003

Jill McGaughy used 192-IgG-SAP (Cat# IT-01)



2004

Michelle Pearson used IB4-SAP (Cat# IT-10)



2005

W.Zhang used Dermorphin-SAP (Cat. #IT-12) and CCK-SAP (Cat. #IT-31)

2006

Neelima Chauhan used mu p75-SAP (Cat# IT-16)

2007

Kevin Pang used Anti-GAT-SAP (Cat# IT-32)

2008

Arshad Khan used Anti-DBH-SAP (Cat# IT-03)

2009

Ai-Jun Li used Leptin-SAP (Cat# IT-47) and NPY-SAP (Cat #IT-28)

2010

Yan Liu used mu p75-SAP (Cat# IT-16)

2011

Melinda Smith and **Sally Krajewski** used NK3-SAP (Cat# IT-63) and Blank-SAP (Cat. #IT-21)

2012

Ko Zushida used Bombesin-SAP (Cat# IT-40)

2013

Damla Khan used Mac-1-SAP (rat) (Cat# IT-33)

Targeting Tools: Top Twenty (Five Each in Four Categories)

Top Five Targeted Toxins

#1. 192-IgG-SAP (192-Saporin) (Cat. #IT-01)
targets cells expressing rat p75^{NTR}

#2. Anti-DBH-SAP (Cat. #IT-03)
targets cells expressing rat dopamine beta-hydroxylase (DBH)

#3. mu p75-SAP (Cat. #IT-16)
targets cells expressing mouse p75^{NTR}

#4. IB4-SAP (Cat. #IT-10)
targets cells expressing α-D-galactopyranoside residues

#5. Mac-1-SAP mouse/human (Cat. #IT-06)
targets cells expressing mouse / human mac-1 (CD11b) receptor

TOP TARGETED TOXIN OF 2013

192-IgG-SAP (192-Saporin) (Cat. #IT-01)
targets cells expressing rat p75^{NTR}

Top Five Antibodies

#1. NGFr (mu p75) Rabbit Polyclonal (Cat. #AB-N01)

#2. NGFr (ME20.4, p75) Mouse Monoclonal (Cat. #AB-N07)

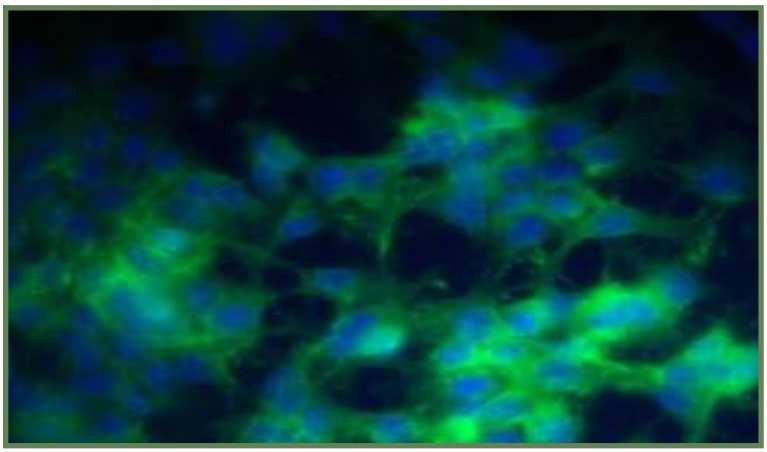
#3. trkA Rabbit Polyclonal (Cat. #AB-N03)

#4. NGFr (mu p75) Rabbit Polyclonal, affinity-purified (Cat. #AB-N01AP)

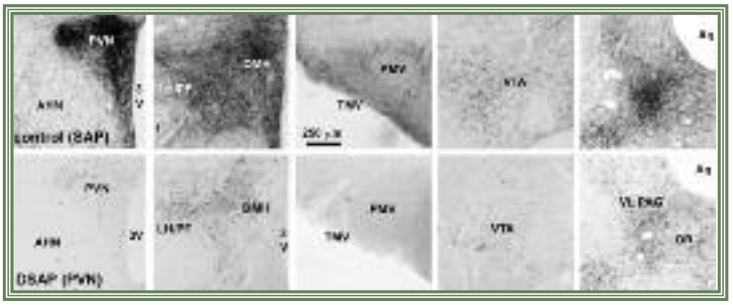
#5. Angiotensin II receptor (AT-2r) Rabbit Polyclonal, affinity-purified (Cat. #AB-N28AP)

TOP ANTIBODY OF 2013

NGFr (ME20.4, p75) Mouse Monoclonal (Cat. #AB-N07)



AB-N01AP. Immunofluorescent staining of NG6 cells, a clone of the NG108-15 fusion of a mouse neuroblastoma and rat glioblastoma. Cells were fixed with paraformaldehyde and blocked prior to staining with primary at 10 µg/ml followed by goat anti-rabbit-FITC secondary at 50 µg/ml and DAPI at 5 µg/ml for nuclear staining. Images were obtained using a 40x objective and a LeicaDM IL fluorescent microscope. NGFr staining is represented in green and nuclear staining is represented in blue.



Lesion with anti-DBH-SAP (DSAP) aimed at the PVN leads to dramatic loss of DBH-labeled innervation in the PVN, but also other parts of the hypothalamus (dorsomedial [DMH], lateral/perifornical [LH/PF], ventral tuberomammillary [TMV] portions), as well as the ventral tegmental area (VTA) and the ventrolateral periaqueductal grey (VLPAG) and dorsal raphe (DR). Telencephalic regions targeted by the locus coeruleus were not affected.
Contributed by Lisa E. Goehler and Ronald P.A. Gaykema

Top Five Secondary Conjugates

#1. Mab-ZAP (Cat. #IT-04)
Uses your primary mouse monoclonal antibody

#2. Streptavidin-ZAP (Cat. #IT-27)
Uses your biotinylated material in order to evaluate the ability of the reagent to internalize upon binding to its receptor

#3. Anti-6 His-ZAP (Cat. #IT-52)
Uses your 6-His-tagged proteins over-expressed in cells

#4. Rab-ZAP (Cat. #IT-05)
Uses your primary rabbit affinity-purified polyclonal IgG antibody to target and eliminate cells

#5. Fab-ZAP rat (Cat. #IT-55)
Uses your primary rat monoclonal IgG antibody

TOP SECONDARY CONJUGATE OF 2013

Fab-ZAP human (Cat. #IT-51)
Uses your primary human monoclonal IgG antibody to target

Top Five in Publications

#1. 192-IgG-SAP (192-Saporin) (Cat. #IT-01)
targets cells expressing rat p75^{NTR}

#2. Anti-DBH-SAP (Cat. #IT-03)
targets cells expressing rat dopamine beta-hydroxylase (DBH)

#3. SSP-SAP (Cat. #IT-11)
targets cells expressing substance P (NK-1) receptor

#4. mu p75-SAP (Cat. #IT-16)
targets cells expressing mouse p75^{NTR}

#5. Streptavidin-ZAP (Cat. #IT-27)
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Targeting Teaser

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

NEXTOUNIRO

ZANYALE

SKROWER

SNOBBEMI

JOINTINCE

What do you wish someone for their 20th anniversary?

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

ANSWER:
MAY YOU ENJOY . . . ○○○○○○ ○○○○ !

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SOLVE the puzzle online with the correct solution by June 30, 2014.

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www.atsbio.com/news/14q2_teaser.html

Jul-Aug-Sep 2014
Volume 15, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

Corticotropin releasing factor-saporin conjugate selectively lesions nucleus incertus.

Contributed by Corinne Liying Lee, Ramamoorthy Rajkumar, Gavin Stewart Dawe, Department of Pharmacology, Yong Loo Lin School of Medicine, National University Health System; Neurobiology and Ageing Programme, Life Sciences Institute, National University of Singapore; and Singapore Institute for Neurotechnology (SINAPSE), Singapore 117456

The nucleus incertus (NI) is a distinct group of cells in the pontine periventricular gray, adjacent to the dorsal tegmental nucleus.[1-3] Interest in this small and distinct group of neurons spiked after it was shown to express high levels of corticotropin releasing hormone receptor 1 (CRF1),[4-5] which suggests a role for the NI in the circuitry of stress responses. While the NI also expresses neuromedin B[6-7] and cholecystokinin,[3,8] expression of relaxin-3 overlaps that of CRF1 and is highly specific and mostly concentrated in the NI. As such, the NI is considered the principle source of relaxin-3 in the mammalian brain and possibly serves as a key point of regulation of the relaxin-3 neural circuitry. The exact function of the NI and relaxin-3 is still largely unknown but the NI has been implicated in the control of multiple neural networks, ranging from the regulation of feeding behavior,[9-11] to the modulation of stress and anxiety[2,12-13] and cognition.[14-16] In our recent study published in *Brain Research*, we demonstrated a method for the selective ablation of CRF1-expressing neurons in the NI using the CRF-SAP conjugate (CRF-SAP; Cat. #IT-13).[17]

While some of the hypothesized functions of the NI are speculations from anatomical

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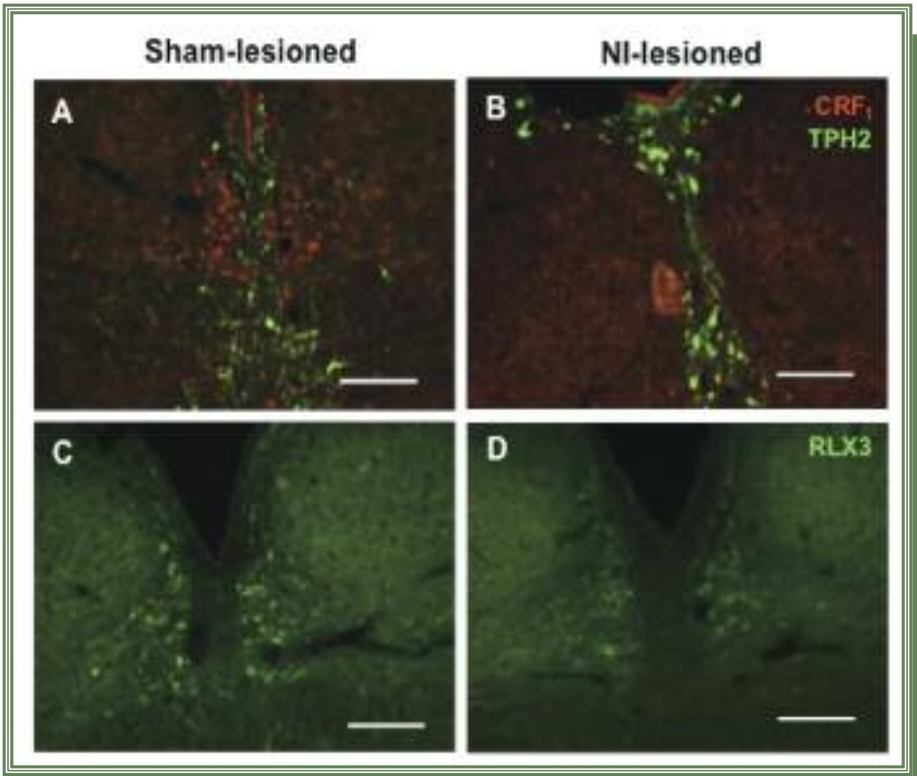


Immunohistochemical analysis of CRF–SAP lesioning of NI neurons. Representative images depict a decrease in CRF1 (B) and relaxin-3 expressing cells (D) in NI-lesioned rats compared to the sham-lesioned group (A and C), respectively. Presence of midline TPH2-positive cells in both (A) sham- and (B) NI-lesioned groups indicated that CRF-SAP selectively targeted only CRF1-positive NI cells. Scale bars are 100 µm.

Inside this issue:

Targeting Topics
Summary of Scientific References 3

Targeting Talk
Questions & Answers 5

Targeting Tools
20th Anniversary Special 7

Targeting Teaser
Word Quiz 8

Newsletter Highlights

- ZAP Antibody Internalization Kit *(page 2)*
- Teaser Winners *(page 6)*
- Recent Scientific References *(page 3)*
- Product Q&A *(page 5)*
- Targeting Teaser Win a Flashlight Pen! *(page 8)*

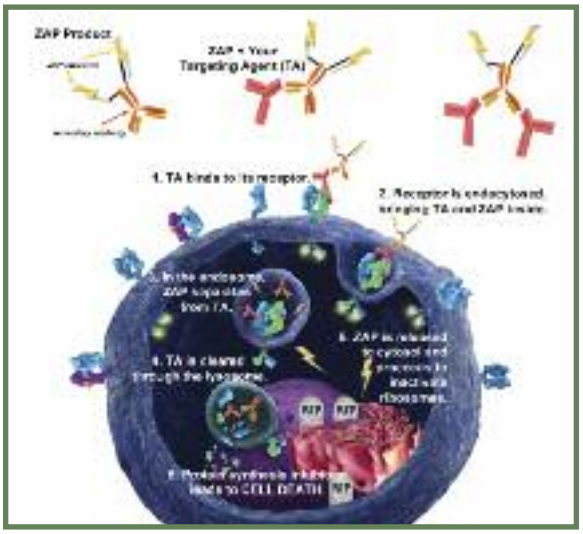
Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

ZAP Antibody Internalization Kit -- How does it work?

Screening large numbers of antibodies for the ability to internalize can be prohibitively expensive in both cost and time. The ZAP Antibody Internalization Kit contains all the components needed for three 96-well plates, or 288 tests. The ability to perform a diagnostic screen that is amenable to high-throughput methods, prior to direct conjugation of those antibodies, is a great cost-benefit in the development of an effective targeted conjugate.

The ZAP Antibody Internalization Kit contains all of the materials needed to screen your antibody. Included, in addition to the selected ZAP products, are controls and XTT developing reagents for the assay. All the user provides are the materials specific to their experiment (the antibody candidate, cells expressing the target, and culture reagents). Recommended protocols for use are detailed in a booklet and on a flash drive provided, and are specific to the particular kit chosen (Whole-ZAP, Fab-ZAP, or FabFc-ZAP). Examples of predicted assay results are also included for comparison; a successful assay provides an EC_{50} useful in determining if the candidate-antibody should be pursued at the next level.



ATS Customer: "Can you tell me, in a simple way, how I can tell if my antibody internalizes using your kit?"

Brian Russell, Product Manager: "The ZAP technology uses the unique properties of Saporin to verify the binding and internalization of your antibody. Saporin is a ribosome inactivating protein with no binding component, so the only way for Saporin to be toxic to cells is if it is escorted inside the cells via interaction with something else that is internalized (i.e. Your antibody). The ZAP kit measures cell death that is directly and specifically resulting from your antibody being internalized."

ATS Customer: "How long does it take to get results?"

Brian Russell, Product Manager: "The whole protocol from start to finish takes 5 days. There is an overnight incubation period for cell acclimation and a 72 hour incubation of the cells with your antibody that also gives the Saporin enough time to cause cell death. The hands-on time is approximately 30-60 minutes on each of 3 days during the 5-day protocol."

ATS Customer: "How many antibodies can I screen at one time?"

Brian Russell, Product Manager: "Each ZAP Kit is designed to test one antibody titrated in sextuplets at 8 concentrations. There are also materials for 1-2 control plates, depending on the kit."

ATS Customer: "Do you have a kit that will work with other materials — such as peptides?"

Brian Russell, Product Manager: "We do have another product, Biotin-Z Kit (COMING SOON), that is designed to work with biotinylated materials. If you have a peptide, or other protein that is biotinylated, you can use the Biotin-Z kit as a way to implement ZAP technology when you are working with materials other than antibodies."

Upcoming Events

Society for Neuroscience
November 15-19, 2014
Washington, DC
Booth #1019

Spring Brain Confeence
March 19-22, 2015
Sedona, AZ
www.SpringBrain.org

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Role for monocyte chemoattractant protein-1 in the induction of chronic muscle pain in the rat.

Alvarez P, Green PG, Levine JD.

Pain 155(6):1161-1167, 2014.

In order to better understand where monocyte chemoattractant protein 1 (MCP-1) fits in the chronic pain landscape the authors performed a series of experiments using antisense and mismatch oligodeoxynucleotides against the MCP-1 receptor in rats. Some animals also received 3.2 µg intrathecal injections of IB4-SAP (Cat. #IT-10). IB4-SAP treatment removed water avoidance stress-induced muscle hyperalgesia, as well as preventing stress-induced hyperalgesic priming that is a usual response to administration of MCP-1. The data indicate that MCP-1 takes action through its receptors on IB4+ nociceptors.

P2Y1 receptor−mediated potentiation of inspiratory motor output in neonatal rat in vitro.

Alvares TS, Revill AL, Huxtable AG, Lorenz CD, Funk GD.

J Physiol Epub, 2014.

P2YR's are metabotrophic purinergic receptors found in some parts of the CNS. A subtype of this receptor excites rhythm generating networks in the preBötzinger complex. In order to better understand the role of these receptors in modulation of motor output the authors used brainstem-spinal cord and medullary slice preparations from neonatal rats to investigate P2Y1R signaling on specific neurons that innervate diaphragm and airway muscles. Anti-NK1r (Cat. #AB-N33AP) at a 1:1000 dilution was used during the immunohistochemistry. The data suggest that loss of purinergic modulation contributes to motoneuron excitability.

Orexin A activates hypoglossal motoneurons and enhances genioglossus muscle activity in rats.

Zhang GH, Liu ZL, Zhang BJ, Geng WY, Song NN, Zhou W, Cao YX, Li SQ, Huang ZL, Shen LL.

Br J Pharmacol Epub, 2014.

Orexin neurons are restricted to the lateral hypothalamus (LH) and are involved in functions such as feeding behavior, energy homeostasis, sleep/wake cycles, and many others. Here the authors investigate orexin control of the genioglossus – the largest upper airway dilator muscle. Rats received bilateral 172 ng injections of orexin-SAP* into the LH. Lesioned animals displayed a significant decrease in genioglossus muscle electromyograms, indicating that orexin neurons are vital to the control of this muscle.

Noradrenaline neuron degeneration contributes to motor impairments and development of L-DOPA-induced dyskinesia in a rat model of Parkinson's disease.

Shin E, Rogers JT, Devoto P, Bjorklund A, Carta M.

Exp Neurol 257C:25-38, 2014.

Although Parkinson's disease is usually associated with loss of dopaminergic neurons in the substantia nigra, post-mortem studies have shown that noradrenergic neurons in the locus coeruleus also degenerate. In this work the authors develop a new Parkinson's disease model by double lesioning with both 6-OHDA into the striatum and 2.5 µg bilateral injections of anti-DBH-SAP (Cat. #IT-03) into the lateral ventricles of rats. Double-lesioned animals performed worse on tests evaluating Parkinson's disease symptoms than those lesioned only with 6-OHDA. The data suggest that Parkinson's disease symptoms reflect the loss of both dopaminergic and noradrenergic neurons in the midbrain.

Role of paraventricular nucleus-projecting norepinephrine/epinephrine neurons in acute and chronic stress.

Flak JN, Myers B, Solomon MB, McKlveen JM, Krause EG, Herman JP.

Eur J Neurosci 39(11):1903-1911, 2014.

Chronic stress can cause dysregulation of the paraventricular nucleus (PVN) of the hypothalamus, resulting in structural and function changes in the neurons involved. There are data indicating that post-stress enhancement of norepinephrine is involved in the processing of chronic stress. In this work the authors investigated the hypothesis that PVN-projecting norepinephrine/epinephrine (NE/E) neurons are necessary for chronic stress-induced drive of the hypothalamic-pituitary-adrenocortical (HPA) axis. Rats received bilateral 8.82 ng injections of anti-DBH-SAP (Cat. #IT-03) into the PVN. Saporin (Cat. #PR-01) was used as a control. Lesioned animals displayed attenuated peak ACTH, indicating that NE/E neurons are required for ACTH release in the HPA axis during chronic stress.

The cholinergic basal forebrain in the ferret and its inputs to the auditory cortex.

Bajo VM, Leach ND, Cordery PM, Nodal FR, King AJ.

Eur J Neurosci Epub, 2014.

The ferret has become a more common animal model in auditory neuroscience. Unlike rodent models, however, anatomical data describing the organization of the basal forebrain cholinergic system and its projections to the auditory cortex have not been well characterized. Using a variety of methods the authors mapped the architecture of the ferret basal forebrain. IHC was done with several antibodies including anti-ChAT (Cat. #AB-N34AP; 1:1000) and anti-NGFr (Cat. #AB-N07; 1:500). Animals also received 17 µg of ME20.4-SAP (Cat. #IT-15) in a total of 17 injections into the ectosylvian gyrus. The results indicate that acetylcholine is most likely involved in modulation of auditory processing.

Role of lateral hypothalamus in two aspects of attention in associative learning.

Wheeler DS, Wan S, Miller A, Angeli N, Adileh B, Hu W, Holland PC.

Eur J Neurosci Epub, 2014.

The lateral hypothalamic (LH) region contains both orexin and melanin-concentrating hormone (MCH) neurons. These neurons are unique to the LH but project throughout the brain. In this work the authors examined the role of the LH in specific attentional aspects of associative learning. Rats received unilateral 500 ng injections of orexin-SAP* into the LH and

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)
were tested in several learning tasks. The lesioned animals displayed impaired behavior that was correlated to the loss of orexin but not MCH neurons.

Effects of hypocretin/orexin cell transplantation on narcoleptic-like sleep behavior in rats.

Arias-Carrion O, Murillo-Rodriguez E.
PLoS One 9(4):e95342, 2014.

In this work the authors examined the effect of orexin cell grafts into the lateral hypothalamus (LH) on narcoleptic-like sleep behavior. Rats received bilateral 490-ng injections of orexin-SAP* into the LH. 21 days post-lesion, the animals then received a graft consisting of dissociated cells from a 8-10 day old rat brain. The narcoleptic-like behavior was reduced in animals receiving the graft, indicating that restoration of some orexin levels may help resolve neurodegeneration.

Effects of noradrenergic denervation by anti-DBH-saporin on behavioral responsivity to l-DOPA in the hemi-parkinsonian rat.

Ostock CY, Lindenbach D, Goldenberg AA, Kampton E, Bishop C.
Behav Brain Res 270C:75-85, 2014.

Dopamine loss is central to Parkinson's disease and is often accompanied by noradrenergic denervation of the locus coeruleus. In this work the authors examined the role this loss plays in L-DOPA therapy using a rat Parkinson's disease model. The rats received 10 µg of anti-DBH-SAP (Cat. #IT-03) into the left lateral ventricle. Loss of norepinephrine (NE) neurons did not affect behavior, but lesioned animals were less responsive to the pro-motor therapeutic effects of L-DOPA.

Descending controls modulate inflammatory joint pain and regulate CXC chemokine and iNOS expression in the dorsal horn.

Carr FB, Géranton SM, Hunt SP.
Mol Pain 10(1):39, 2014.

Peripheral joint pathology in conditions such as osteoarthritis does not always correlate to the amount of pain experienced, indicating that chronic pain is present. The role of descending facilitation in this form of chronic pain has not been investigated. The authors examined the role of mu opioid receptor-expressing cells in the rostral ventral medulla (RVM) in behavioral hypersensitivity seen in joint pain models. Rats received 1.5 pmol of Dermorphin-SAP (Cat. #IT-12) into the RVM. Lesioned animals displayed prolonged attenuation of hypersensitivity, and altered expression of several genes was detected by qPCR, indicating that descending facilitation in the RVM is involved in joint pain behavior.

Cholinergic Immunotoxin 192 IgG - SAPORIN Alters Subicular Theta-Gamma Activity and Impairs Spatial Learning in Rats.

Rastogi S, Unni S, Sharma S, Rao Laxmi T, Kutty BM.
Neurobiol Learn Mem Epub, 2014.

The authors investigated the role of the subiculum in spatial informational processing, specifically cholinergic modulation of subicular theta-gamma activity. Rats received 50-ng injections of 192-IgG-SAP (Cat. #IT-01) into the ventral subiculum. Lesioned animals displayed altered theta and gamma activity as well as impaired spatial learning. The hippocampal cholinergic innervations remained intact, indicating that cholinergic modulation of theta-gamma activity in the subiculum plays an important role in spatial information processing.

Depletion of inflammatory dendritic cells with anti-CD209 conjugated to saporin toxin.

Alonso MN, Gregorio JG, Davidson MG, Gonzalez JC, Engleman EG.
Immunol Res 58(2-3):374-377, 2014.

Monocyte activity is critical in inflammatory responses, however the accumulation of inflammatory dendritic cells (DC) that are derived from monocytes can cause pathogenesis and persistence of some inflammatory diseases. The authors used a mouse model in which injection of lipopolysaccharides cause an abundance of inflammatory DC's. The mice then received a biotinylated antibody against CD209 coupled to streptavidin-ZAP (Cat. #IT-27). Mice receiving the conjugate had lower levels of CD209-positive DC's, indicating the potential for this strategy in reducing the DC effect during inflammatory disease.

Armodafinil-induced wakefulness in animals with ventrolateral preoptic lesions.

Vetrivelan R, Saper CB, Fuller PM.
Nat Sci Sleep 6:57-63, 2014.

Excessive daytime sleepiness is often treated with modafinil. Armodafinil, the R-isomer of modafinil, has been introduced for clinical use, but little is known about the cellular pathway targeted by these drugs. The authors examined whether armodafinil inhibits the ventrolateral preoptic nucleus (VLPO). Rats received 200-ng injections of orexin-SAP* into the VLPO, followed by administration of armodafinil. Lesioned animals displayed increased wakefulness similar to control animals, indicating that armodafinil and modafinil do not act along the VLPO neurons.

The combinational use of CRISPR/Cas9-based gene editing and targeted toxin technology enables efficient biallelic knockout of the alpha-1,3-galactosyltransferase gene in porcine embryonic fibroblasts.

Sato M, Miyoshi K, Nagao Y, Nishi Y, Ohtsuka M, Nakamura S, Sakurai T, Watanabe S.
Xenotransplantation 21(3):291-300, 2014.

In this work the authors destroyed the function of a porcine gene involved in α-Gal production. α-Gal is involved in hyperacute rejection of pig tissues in humans. After targeting the gene, 10^5 cells were incubated with 0.5 µg of IB4-SAP (Cat. #IT-10) in order to eliminate any remaining α-Gal expressing cells. Surviving cells lacked α-Gal and are candidates for the creation of knockout cloned piglets.

New versions of Orexin-SAP are now in development and production. If you are interested in testing Orexin-SAP and/or orexin receptor antibodies, please contact us.

Targeting Talk: Product Q&A

Q: I ordered the Streptavidin-ZAP and had my antibody biotinylated a couple of months ago. I am ready to begin the first round of experiments to determine the concentration needed for the secondary. How much of the biotinylated antibody should I put to combine with the streptavidin for intravitreal injections? Can you please send me a protocol for how to determine the ratio of primary to secondary?

A: The streptavidin-ZAP should be mixed with the biotinylated material at an equimolar concentration. The streptavidin-ZAP you ordered should have included a data sheet which gives the protein concentration and molecular weight, which you would use to determine the molar concentration. We have a calculator page on our website which can help with this if needed.

http://www.atsbio.com/calculations.html

Here is a link to our references page for Streptavidin-ZAP which lists the different publications using this product, and I have also listed a specific publication using streptavidin-ZAP combined with a biotinylated antibody being used in intravitreal injections. The reference describes in detail the quantities they tried.

http://www.atsbio.com/reference/it27.html

Ren C, Luan L, Wui-Man Lau B, Huang X, Yang J, Zhou Y, Wu X, Gao J, Pickard GE, So KF, Pu M. (2013) Direct Retino-Raphe Projection Alters Serotonergic Tone and Affective Behavior. *Neuropsychopharmacology* 38(7):1163-1175.

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Targeted Toxins

192-IgG-SAP (192-Saporin)
Acetylated LDL-SAP
Anti-CD103-SAP
Anti-CD25-SAP, human
Anti-ChAT-SAP
Anti-DAT-SAP
Anti-DBH-SAP
Anti-GAT-SAP
Anti-SERT-SAP
Bombesin-SAP
CCK-SAP
CRF-SAP
CTB-SAP
Dermorphin-SAP
Dyno-SAP
FGF-SAP
Galanin-SAP
IB4-SAP
Leptin-SAP
Mac-1-SAP
ME20.4-SAP
Melanopsin-SAP
mu p75-SAP
Neurotensin-CTA
Neurotensin-SAP
Nppb-SAP
NK3-SAP
NPY-SAP
Orexin-SAP
OX7-SAP
Oxytocin-SAP
Neurotensin-SAP
SP-CTA
Stable/Specific Substance P-SAP
vGAT-SAP

Control Conjugates

Secondary Antibody Conjugates

Streptavidin-ZAP

Antibodies

Fluorescent Conjugates

Alexa488-labeled 192-IgG
Cy3-labeled 192-IgG
Cy3-labeled Anti-murine NGFr
FITC-labeled Anti-SAP
FITC-labeled Goat Anti-Rabbit IgG
FITC-labeled Goat Anti-Mouse IgG

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IB4
Saporin
SERT Peptide

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Chick-ZAP, Rabbit IgG-SAP, Saporin	**KIT-62-Z**
Goat-ZAP, Rabbit IgG-SAP, Saporin	**KIT-36-Z**
gPIG-ZAP, Goat IgG-SAP, Saporin	**KIT-64-Z**
Hug-M-ZAP, Goat IgG-SAP, Saporin	**KIT-43-Z**
Hum-ZAP, Goat IgG-SAP, Saporin	**KIT-22-Z**
Mab-ZAP, Goat IgG-SAP, Saporin	**KIT-04-Z**
Rab-ZAP, Goat IgG-SAP, Saporin	**KIT-05-Z**
Rat-ZAP, Goat IgG-SAP, Saporin	**KIT-26-Z**
Fab-ZAP human, Fab-IgG-SAP, Saporin	**KIT-51-Z**
Fab-ZAP mouse, Fab-IgG-SAP, Saporin	**KIT-48-Z**
Fab-ZAP rabbit, Fab-IgG-SAP, Saporin	**KIT-57-Z**
Fab-ZAP rat, Fab-IgG-SAP, Saporin	**KIT-55-Z**
FabFc-ZAP human, Fab-IgG-SAP, Saporin	**KIT-65-Z**

View the videos online. *(http://atsbio.com/zapkit)*

CRF-SAP selectively lesions nucleus incertus.

(continued from page 1)

mapping of its vast connections to the rest of the brain, most of the experimental evidence to date stems from studies using relaxin-3 related peptides, studies of neural activation based on behavioral activity, electrical stimulation or infusion of CRF. To study the role of the NI, bilateral injections of 86 ng/site of CRF-SAP were made into the NI to selectively ablate the CRF1-expressing neurons in the NI. As a control, sham lesions were made by infusion of Blank-SAP (Cat #IT-21). Verification of the lesion after 14 days of recovery, revealed a significant and specific reduction in CRF1 and relaxin-3 positive cells in the NI (See Cover Figure). These NI-lesioned rats also exhibited a significantly greater freezing period when compared to sham-lesioned rats when tested in a cued fear conditioning paradigm.

Our results suggest that CRF-SAP selectively targets and kills CRF1-expressing cells in the NI, consistently decreasing relaxin-3 levels in the NI and one of its known projection targets, the medial septum (MS). Taken together, these data indicate that this CRF1 receptor-targeted lesion model is a valuable tool for studying the relaxin-3 circuitry in the brain. The clear behavioral deficit observed also supports the use of CRF-SAP to selectively perturb the NI and thus provides an additional approach to study the NI and the relaxin-3 system in behavioral neuroscience. The approach to selectively lesion relaxin-3 positive cells in the NI using CRF-SAP expands the spectrum of use of targeted toxins to study the function of discrete neuron subgroups in other brain structures.

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Targeting Teaser Solution

The solution to the puzzle was:

Jumbles: NEUROTOXIN
ANALYZE
WORKERS
BOMBESIN
INJECTION

What do you wish someone for their 20th anniversary?

Answer: TWENTY MORE!

Solve this quarter's teaser at www.ATSbio.com/news/14q3_teaser.html

Congratulations to the puzzle solvers from last quarter. Each winner has received a tote bag featuring the 25th Annual SBC.

LAST QUARTER'S WINNERS: Thea Marlinga, Liberty, IL * Glenn H. Kageyama, Cal Poly Pomona Univ, Pomona, CA * Jheem D. Medh, California State Univ, Northridge, CA * Seto Chice, SUNY Downstate Medical Center, Brooklyn NY * Bill Henry, Lifespan Tech, Rhode Island Hospital, Providence, RI * Daniel Pekala, Charles River Immunopathology, Frederick, MD * Richard Fuerstenberg, R&D Systems, Inc, Minneapolis, MN * Cory Kim, Rancho Santa Margarita, CA

Targeting Tools: Top Five Over Twenty Years -- 20% off!

Top Five Targeted Toxins

192-IgG-SAP (192-Saporin) (Cat. #IT-01)
targets cells expressing rat p75^{NTR}

Anti-DBH-SAP (Cat. #IT-03)
targets cells expressing rat dopamine beta-hydroxylase (DBH)

mu p75-SAP (Cat. #IT-16)
targets cells expressing mouse p75^{NTR}

IB4-SAP (Cat. #IT-10)
targets cells expressing α-D-galactopyranoside residues

Mac-1-SAP mouse/human (Cat. #IT-06)
targets cells expressing mouse / human mac-1 (CD11b) receptor

Top Five Antibodies

NGFr (mu p75) Rabbit Polyclonal (Cat. #AB-N01)
recognizes the p75NTR (low affinity neurotrophin receptor) in mouse

NGFr (ME20.4, p75) Mouse Monoclonal (Cat. #AB-N07)
recognizes the p75NTR in human, primate, rabbit, sheep, dog, cat, and pig

trkA Rabbit Polyclonal (Cat. #AB-N03)
recognizes the trkA (high affinity nerve growth factor receptor) in rat

NGFr (mu p75) Rabbit Polyclonal, affinity-purified (Cat. #AB-N01AP)
recognizes the p75NTR in mouse

Angiotensin II receptor (AT-2r) Rabbit Polyclonal, affinity-purified (Cat. #AB-N28AP))
recognizes the Angiotensin II type 2 receptor (AT-2) in rat

Top Five Secondary Conjugates

Mab-ZAP (Cat. #IT-04)
uses your primary mouse monoclonal antibody

Streptavidin-ZAP (Cat. #IT-27)
uses your biotinylated material in order to evaluate the ability of the reagent to internalize upon binding to its receptor

Anti-6 His-ZAP (Cat. #IT-52)
uses your 6-His-tagged proteins over-expressed in cells

Rab-ZAP (Cat. #IT-05)
uses your primary rabbit affinity-purified polyclonal IgG antibody to target and eliminate cells

Fab-ZAP rat (Cat. #IT-55)
uses your primary rat monoclonal IgG antibody

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§ or anything recognized on the cell surface and internalized.

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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 the cover story**, one letter to each block, to solve the puzzle.

CARESINGED

HIBORAVE

TONALABI

KNALB

TURYRICCI

What did the Lego Scientist call DNA?

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

ANSWER:
A LABORATORY . . . !

WIN!

SOLVE the puzzle online with the correct solution by September 30, 2014.

WIN an ATS flashlight pen!

www.atsbio.com/news/14q3_teaser.html

Oct-Nov-Dec 2014
Volume 15, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Cancer Pain Relief in Pet Dogs has Direct Translation into Human Chronic Pain Conditions

Based on the recent publication by: *Brown DC, Agnello K. (2013) Intrathecal substance p-saporin in the dog: efficacy in bone cancer pain. Anesthesiology 119(5):1178-1185* and the October 2013 press release by the American Society of Anesthesiologists, *"Man's best friends' chronic pain relieved with new treatment, study finds: Findings could be useful to human cancer patients.*

A single injection eased severe, chronic pain caused by late-stage bone cancer in dogs, according to a study in the November issue of *Anesthesiology*. Dogs with bone cancer that received a neurotoxin injection had significantly more pain relief than those that got standard care without the injection. "Dogs are part of the family and we do everything we can to relieve them of pain and discomfort when they are sick," said Dorothy Cimino Brown, D.V.M., School of Veterinary Medicine, University of Pennsylvania, Philadelphia. "In addition to sharing emotional attachments with our dogs, humans share many of the same ailments our pets suffer when fighting cancer. By studying the positive pain relief this treatment afforded dogs, we are hopeful it may also be effective for humans." The evolution of bone cancer pain in dogs parallels what occurs in humans, with the frequency and intensity of pain increasing over weeks and months. As the cancer advances, both canine and human patients experience life-altering pain, which greatly affects their daily activities and quality of life. The standard treatment for dogs with late-stage bone cancer can include opioids, steroids, and palliative radiation. All of these treatments can have negative side effects.

The positive pain-relieving effect that SP-SAP had was significant in the veterinary study in pet dogs with bone cancer. Not only does this provide promising data for canine patients suffering from cancer, it also gives credence to the successful use of SP-SAP for chronic pain control in humans.
(See Clinical Trial update on Page 6).

The owners of 70 dogs enrolled their pets in this study. Half the dogs received an injection of a neurotoxin, called substance P-saporin (SP-SAP), as well as standard care. The other half (i.e., the control group) received standard care without the neurotoxin injection. The average age of the dogs was between 8 and 9 years and their average weight was 90 pounds. Multiple breeds participated in the study, including: Rottweilers, Labrador Retrievers, Golden Retrievers and mixed breeds.

Neurotoxins are historically known for the disease they can cause, such as botulism, according to Dr. Brown. More recently, however, scientists have learned to harness properties of neurotoxins for positive uses. For example, Botox is used to eliminate wrinkles and SP-SAP is used to decrease pain. The SP portion of the neurotoxin works by attaching to a pain-transmitting nerve and then the "SAP" part gets inside the nerve and causes it to die.

Within six weeks of beginning the study, 74 percent (26) of the dogs in the control group needed to be "unblinded" (in other words, their status in the study revealed) and their pain relief

(continued on page 6)

Inside this issue:

Targeting Topics
Scientific References 3

Targeting Talk
Questions & Answers 5

Targeting Tools
NEW - StrepZAP kit 7

Targeting Teaser
Word Quiz 8

Highlights

- Otis Gets His Wag Back! *(pg 6)*
- Teaser Winners *(pg 5)*
- Recent Scientific References *(pg 3)*
- SfN 2014 Survey *(pg 2)*
- Targeting Teaser
  Win a 2015 Calendar!
  (page 8)

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Visit our booth at the Society for Neuroscience and Choose Your Gift!

This year, ATS will be conducting a brief iPad-based survey at the Society for Neuroscience meeting in Washington, DC. Your responses regarding antibody screening, internalization kits, and what configurations best meet your needs will enable us to provide you with the perfect ZAP kit.

Spend five minutes to select multiple choice answers and select the gift of your choice!

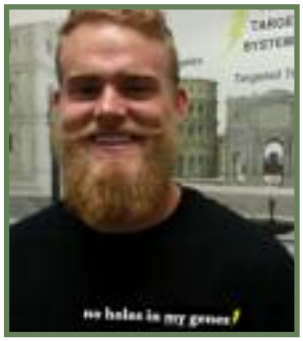
Choice #1. A Knockout T-shirt

The back of the Knockout T-shirts.

Brian with
"Like Knockouts?"

ATS employees model the new Knockout T-shirts. Left to Right: Jason Nathanson, Patrick Shramm, Chelsea Friedman, Brian Russell, Tom Cobb, Matt Kohls

Matt with
"Knock 'em Dead!"

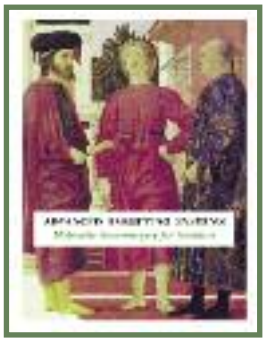


Patrick with
"No holes in my genes!"

Choice #2. A Renaissance Art Poster (18" x 36")

Lovingly referred to by ATS as "The Three Guys," this is an extract from a painting by Italian artist Piero della Francesca.

Choice #3. Surprise!



Society for Neuroscience
November 15-19, 2014
Washington, DC
Booth #1019

Upcoming Events

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

MOUSE, RAT, BOVINE AND HUMAN IMMUNOGLOBULINS G QUANTIFICATION BY ELISA

ATS is pleased to announce a partnership with RD-Biotech to provide FastELISA kits to our customers worldwide. These kits are a natural complement to the ZAP Antibody Internalization Kits. For anyone looking to produce and screen monoclonal antibodies, FastELISA kits are user-friendly and optimized for monoclonal antibody production monitoring, clone selection, murine IgG isotyping, and contaminant detection.

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Mouse immunoglobulin isotyping kit *(Cat. #RDB-01)*
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Rat IgG quantification kit *(Cat. #RDB-03)*
Human IgG quantification kit *(Cat. #RDB-04)*
Bovine IgG quantification kit *(Cat. #RDB-05)*
Protein A quantification kit *(Cat. #RDB-06)*

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Activated Macrophages Create Lineage-specific Microenvironments for Pancreatic Acinar- and beta-cell Regeneration in Mice.

Criscimanna A, Coudriet GM, Gittes GK, Piganelli JD, Esni F.

Gastroenterology Epub2014.

In response to tissue damage or infection, monocytes are recruited to the injured area and differentiate into macrophages. These macrophages can perform different functions depending on the tissue type. The specific differentiation macrophages undergo in response to their environment is called polarization. The authors used a mouse pancreatic lesion model to examine the polarization of macrophages into the two distinct states known, M1 and M2. Mice received 20 µg of Mac-1-SAP mouse (Cat. #IT-06) in a tail vein injection following a pancreatic lesion, and were sacrificed on various days post-injection in order to evaluate macrophage presence at different response stages. The results demonstrate that various aspects of macrophage polarization are required for pancreatic regeneration.

Prolyl hydroxylation by EglN2 destabilizes FOXO3a by blocking its interaction with the USP9x deubiquitinase.

Zheng X, Zhai B, Koivunen P, Shin SJ, Lu G, Liu J, Geisen C, Chakraborty AA, Moslehi JJ, Smalley DM, Wei X, Chen X, Chen Z, Beres JM, Zhang J, Tsao JL, Brenner MC, Zhang Y, Fan C, DePinho RA, Paik J, Gygi SP, Kaelin WGJ, Zhang Q.

Genes Dev 28(13):1429-1444, 2014.

Members of the FOXO family are thought to act as tumor suppressor genes. In this work the authors investigated the hydroxylation of FOXO3a by EglN2. This hydroxylation pushes FOXO3a toward a protosomal degradation pathway. Loss of FOXO3a in turn allows the accumulation of Cyclin D1, which has been found to be overexpressed in some breast cancers. Some of the data were generated using immunoblots with anti-transhydroxylated proline (Cat. #AB-T044).

New versions of Orexin-SAP are now in development and production. If you are interested in testing Orexin-SAP and/or orexin receptor antibodies, please contact us.

Cross-Inhibition of NMBR and GRPR Signaling Maintains Normal Histaminergic Itch Transmission.

Zhao ZQ, Wan L, Liu XY, Huo FQ, Li H, Barry DM, Krieger S, Kim S, Liu ZC, Xu J, Rogers BE, Li YQ, Chen ZF.

J Neurosci 34(37):12402-12414, 2014.

After itch detection, the itch pathway moves through an array of G-protein coupled receptors and transient receptor potential channels in dorsal root ganglion neurons into dorsal horn neurons which integrate and transduce these signals, sending them to the somatosensory cortex. The purpose of this work is to clarify whether gastrin-releasing peptide (GRP) or B-type natriuretic peptide regulates histaminergic itch. Several strains of knockout mice received 200, 300, or 400 ng intrathecal injections of bombesin-SAP (Cat. #IT-40). Blank-SAP (Cat. #IT-21) was used as a control. The data further define the respective functions of the neuromedin B receptor and GRP receptor in itch, and reveals a working relationship between the different interneuron populations.

Microglial VPAC1R mediates a novel mechanism of neuroimmune-modulation of hippocampal precursor cells via IL-4 release.

Nunan R, Sivasathiaseelan H, Khan D, Zaben M, Gray W.

Glia 62(8):1313-1327, 2014.

Postnatal and adult learning and memory require hippocampal neurogenesis. Cognitive dysfunction is frequently accompanied by neuroinflammatory pathogenesis, but the pathways by which the immune system affects neurogenesis are unclear. In this work the authors depleted microglia from primary hippocampal cultures by incubating the cells with 100 µg/ml Mac-1-SAP rat (Cat. #IT-33) for 24 hours. The hippocampal cells were then washed and cultured for further experiments. It was found that neural stem/progenitor cells had reduced survival and proliferation in cultures treated with Mac-1-SAP. These data sketch out the framework of an immune-neuronal pathway important in the regulation of hippocampal neurogenesis.

Light-Triggered, Efficient Cytosolic Release of IM7-Saporin Targeting the Putative Cancer Stem Cell Marker CD44 by Photochemical Internalization.

Bostad M, Kausberg M, Weyergang A, Olsen CE, Berg K, Hogset A, Selbo PK.

Mol Pharm 11(8):2764-2776, 2014.

CD44 is known as a common cancer stem cell (CSC) marker. Given that CSC's seem to have the ability to resist many therapeutic agents, the authors investigated the use of photochemical internalization (PCI) while targeting CD44-expressing CSC's. An immunotoxin was constructed by biotinylating a pan CD44 antibody and combining it with Streptavidin-ZAP (Cat. #IT-27*) at a 4:1 biotinylated antibody to Streptavidin-ZAP molar ratio. Various cancer cell lines were incubated with the toxin at a concentration of 0.825 nM. The toxin showed specific cytotoxicity to CD44-expressing cell lines, demonstrating the efficacy of PCI in conjunction with targeted toxins to treat some cancers.

****See new Biotin-Z Kits on Page 7***

Role of the cerebrospinal fluid-contacting nucleus in the descending inhibition of spinal pain transmission.

Liu H, Yan WW, Lu XX, Zhang XL, Wei JQ, Wang XY, Wang T, Wu T, Cao J, Shao CJ, Zhou F, Zhang HX, Zhang P, Zang T, Lu XF, Cao JL, Ding HL, Zhang LC.

Exp Neurol 261C:475-485, 2014.

The first synapse in the pain pathway is in the spinal dorsal horn, and several sites are involved in the descending control of pain. Previous studies have suggested that cerebrospinal fluid-contacting neurons may facilitate signal transmission and substance transport between the brain parenchyma and the CSF, including processes that modulate pain transmission. The authors administered CTB-SAP (Cat. #IT-14) into the right lateral ventricle of rats. Saporin (Cat. #PR-01) was

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

used as a control. The results indicate that the 5-HT pathway contacting the CSF is an important piece in the descending inhibitory system controlling spinal transmission of pain.

cGMP-dependent protein kinase 1-alpha associates with the antidepressant-sensitive serotonin transporter and dictates rapid modulation of serotonin uptake.

Steiner JA CAMD, Wright J, Mattheis HJF, Prasad HC, Nickl CK, Dostmann WR, Cuchanan CC, Corbin JD, Francis SH, Blakely RD.

Molecular Brain Epub2014.

The neurotransmitter serotonin fulfills an important modulatory role in a wide range of brain functions including mood, appetite, sexual behavior, and reward. Serotonin transporters (SERT) are involved in the inactivation of synaptic serotonin, as well as serotonin recycling, which is critical to the maintenance of neuronal serotonin stores. In this work the authors examined how neuronal A3 adenosine receptor activation can enhance presynaptic serotonin transport *in vitro* as well as SERT-mediated clearance *in vivo*. The *in vitro* experiments included immunohistochemistry with anti-SERT (Cat. #AB-N40) on RN46A cells at a 1:500 dilution.

GABAergic neurons in the medial septum-diagonal band of Broca (MSDB) are important for acquisition of the classically conditioned eyeblink response.

Roland JJ, Janke KL, Servatius RJ, Pang KC.

Brain Struct Funct 219(4):1231-1237, 2014.

The medial septum and vertical limb of the diagonal band of Broca (MSDB) are both important for learning and memory. There are strong connections between these two areas, and damage to one or the other can result in differing dysfunctions. The authors investigated how damage to GABAergic neurons in the MSDB affect acquisition of delay classical conditioning of the eyeblink response (CCER). Rats received 162 ng of GAT-1-SAP (Cat. #IT-32) into the medial septum and 130 ng of GAT-1-SAP into each diagonal band. Treated animals displayed impaired initial acquisition of the eyeblink response, indicating that MSDB GABAergic neurons modulate delay CCER – a task that is not dependent on the hippocampus.

Immunohistochemical Localization of AT1a, AT1b, and AT2 Angiotensin II Receptor Subtypes in the Rat Adrenal, Pituitary, and Brain with a Perspective Commentary.

Premer C, Lamondin C, Mitzey A, Speth RC, Brownfield MS.

Int J Hypertens 2013:175428, 2013.

Angiotensin II is a peptide involved in blood pressure, thirst, and sodium appetite in the brain. It also stimulates aldosterone secretion from the adrenal zona glomerulosa and epinephrine secretion from the adrenal medulla. In order to differentiate between the 3 receptor subtypes for this peptide, subtype-specific antibodies were generated for the AT-1Ar (Cat. #AB-N25AP), AT-1Br (Cat. #AB-N26AP), and AT-2r (AB-N28AP). The antibodies were used in western blotting at a 1:500 dilution, immunohistochemistry (AB-N25AP and AB-N26AP at 1:500, AB-N28AP at 1:2000), and immunoelectron microscopy (at a 1:500 dilution). The results demonstrate that these antibodies are well suited to delineate between angiotensin II receptor subtypes in the brain.

Targeted damage of the cerebrospinal fluid-contacting nucleus contributes to the pain behavior and the expression of 5-HT and c-Fos in the spinal dorsal horn of rats.

Cao J WT, Zhang LC.

Zhongguo Ying Yong Sheng Li Xue Za Zhi 30(3):218-222, 2014. [Article in Chinese]

Pain threshold, 5-hydroxytryptamine (5-HT) expression, and c-Fos expression were measured in rats after treatment with CTB-SAP (Cat. #IT-14). Use of CTB-SAP reduced the number of neurons in the cerebrospinal fluid (CSF)-contacting nucleus over time until no neurons could be detected by the 10th day post-injection. 5-HT and c-Fos expression in the spinal dorsal horn gradually increased, and was negatively correlated with the pain threshold. The data indicate that neurons in the CSF-contacting nucleus are involved in pain regulation, and that expression of 5-HT and c-Fos is part of this regulatory pathway.

Recent Articles using Streptavidin-ZAP (Cat. #IT-27)

Alonso MN, Gregorio JG, Davidson MG, Gonzalez JC, Engleman EG. (2014) Depletion of inflammatory dendritic cells with anti-CD209 conjugated to saporin toxin. *Immunol Res* 58(2-3):374-377.

Lund K, Bostad M, Skarpen E, Braunagel M, Krauss S, Duncan A, Hogset A, Selbo P. (2014) The novel EpCAM-targeting monoclonal antibody 3-17I linked to saporin is highly cytotoxic after photochemical internalization in breast, pancreas and colon cancer cell lines. *MAbs* 6(4):1038-50.

Burgos-Ojeda D, McLean K, Bai S, Pulaski H, Gong Y, Silva I, Skorecki K, Tzukerman M, Buckanovich RJ. (2013) A Novel Model for Evaluating Therapies Targeting Human Tumor Vasculature and Human Cancer Stem-like Cells. *Cancer Res* 73(12):3555-3565.

Bostad M, Berg K, Hogset A, Skarpen E, Stenmark H, Selbo PK. (2013) Photochemical internalization (PCI) of immunotoxins targeting CD133 is specific and highly potent at femtomolar levels in cells with cancer stem cell properties. *J Control Release* 168(3):317-326.

Hess SM, Young EF, Miller KR, Vincent BG, Buntzman AS, Collins EJ, Frelinger JA, Hess PR. (2013) Deletion of naive T cells recognizing the minor histocompatibility antigen HY with toxin-coupled peptide-MHC class I tetramers inhibits cognate CTL responses and alters immunodominance. *Transpl Immunol* 29(1-4):138-145.

Ren C, Luan L, Wui-Man Lau B, Huang X, Yang J, Zhou Y, Wu X, Gao J, Pickard GE, So KF, Pu M. (2013) Direct Retino-Raphe Projection Alters Serotonergic Tone and Affective Behavior. *Neuropsychopharmacol* 38(7):1163-1175.

Stratford EW, Bostad M, Castro R, Skarpen E, Berg K, Hogset A, Myklebost O, Selbo PK. (2013) Photochemical internalization of CD133-targeting immunotoxins efficiently depletes sarcoma cells with stem-like properties and reduces tumorigenicity. *Biochim Biophys Acta* 1830(8):4235-4243.

See new Biotin-Z Kits on Page 7

Targeting Talk: Product Q&A

Q: I ordered a control conjugate to use alongside my targeted conjugate, but the two products are at different concentrations. How much control conjugate should I use?

A: Conjugate products are often of differing protein concentrations, meaning dilution of one is usually necessary to ensure comparable amounts of control conjugate and targeted conjugate are used. This adjustment can be done on a molar basis or a protein concentration basis. The data sheet shipped with each Advanced Targeting System conjugate specifies the molecular weight of the product. There are various calculators available on the ATS web site:
(http://www.atsbio.com/calculations.html).
By using these tools, calculations can be done that will ensure the same number of molecules of both control and targeted conjugate are used in your experiment. Alternatively, if the molecular weights of the two products are similar, calculations can be done to use the same amount of control protein as targeted conjugate protein in your experiment.

Q: I have been using your ZAP Antibody Internalization Kit. It is working well for me, but I can only test one antibody at a time. Do you offer the ZAP kit in larger sizes?

A: We do offer kits with sufficient components to test multiple antibody candidates. We offer "Z4" and "Z10" sizes of kits that include all of the same consumable components of the original ZAP kit in quantities sufficient to test 4 or 10 antibodies, respectively. While the included recommended protocol is identical to the original ZAP kit, the added materials provide an opportunity for the experienced researcher to streamline their experiment by testing multiple antibody candidates at one time.

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)

ZAP Kits

ZAP Antibody Internalization Kit
(for in vitro *use)*
Secondary antibody conjugate kits contain all the components needed to screen your antibody. Available in multiple species as well as whole IgG and Fab IgG secondary conjugates

ZAP Biotin-Z Kit
(for in vivo *use)*
Biotin-Z kit (Streptavidin-ZAP) contains all the components needed to screen your biotinylated materials.

Targeting Teaser Solution

The solution to the puzzle was:

Jumbles: DECREASING
BEHAVIOR
ABLATION
BLANK
CIRCUITRY

What did the LEGO scientist call DNA?

Answer: BUILDING BLOCK!

Solve this quarter's teaser at www.ATSbio.com/news/14q4_teaser.html

Congratulations to the puzzle solvers from last quarter. Each winner has received an ATS flashlight pen.

LAST QUARTER'S WINNERS: Glenn H. Kageyama, Cal Poly Pomona Univ, Pomona, CA * Peter Syapin, Texas Tech Univ Health Sciences Center * Kristen Phend, Univ North Carolina Chapel Hill * Bill Henry, Rhode Island Hospital Surgical Research * Michelle Connole, Harvard Univ/NEPRC * Roger Guillemin, Salk Institute * Daniel Pekala, Charles River Labs * Debbie Nation, Central Methodist Univ * Norma Huff, Univ California San Diego * Bob Lamm, Univ Washington

Otis Gets His Wag Back!

Dog with bone cancer jumps for joy over new treatment that made him pain-free after one dose!

Otis, the golden retriever, was in his golden years when bone cancer pain threatened to shorten his life. As a participant in a clinical trial aimed at pet dogs, he was given a single treatment with a pain therapy called SP-SAP that was developed by Advanced Targeting Systems (ATS).

The story of Otis's relief from cancer pain is well documented in a video accessible at www.ATSbio.com/vet. "Otis Gets His Wag Back" shows Otis before treatment and after. It is clear from Otis's body language and gait there is change for the better, while the tumor has doubled in size.

Bone cancer at advanced stages often leads to animals being euthanized for pain-related issues. There are better options. Pain shouldn't be a life-threatening disease for your pet. SP-SAP prevents chronic pain signals from reaching the brain. The pet's brain no longer knows it is in pain from the cancer, but can still feel normal acute pain that can be treated with traditional pain.

A study sponsored by ATS has promising results that were published in the November 2013 issue of the journal *Anesthesiology*. Pet dogs receiving SP-SAP displayed significantly less pain than dogs receiving traditional pain care, with no visible side effects and an improved quality of life.

With the hope of translating these results to humans, a clinical trial testing SP-SAP on terminal cancer patients has begun. Early results are promising, but additional patients are needed. See http://clinicaltrials.gov/show/NCT02036281 for more information.

SP-SAP Facts:

- Permanent relief from cancer pain
- Single injectable dose
- Successfully tested in dogs
- In human clinical trials

For more information, contact Brian Russell, Vice President of Business Development (brian@targetedtoxins.com).

SP-SAP for Treatment of Cancer Pain

(continued from page 1)

regimen adjusted. This is compared to just 24 percent of the dogs (8) needing adjustment to pain relief regimen in the group that received SP-SAP treatment. This was a statistically significant difference.

Other study results included a 6 percent increase in pain severity scores for dogs in the control group, while the dogs in the SP-SAP group had no change in pain severity score. In addition, the dogs in the control group had an 8 percent increase in how pain interferes with their typical activities, while the SP-SAP dogs had a 5 percent improvement in this pain impact score. Finally, one dog in the control group responded with lessened lameness, while 6 dogs in the SP-SAP group became less lame. While these secondary study results were not statistically significant because they were only assessed two weeks after injection, they are promising.

"The overriding goal of this research is to identify breakthroughs in managing chronic pain in both people and dogs by taking advantage of the fact that pets, through the course of their natural lives, develop many of the same medical conditions causing chronic pain that develop in people," said Dr. Brown. "Additionally we can 'measure' this pain in companion animals like we do in people, quantifying severity and impact on routine activities, mobility and sleep."

The positive pain relieving effect that SP-SAP had was significant, according to the study. It both provides promising data for canine patients suffering from cancer and encourages further research into the use of SP-SAP for chronic pain control in humans.

Targeting Tools: New Internalization Kit and Beta-Testing

ZAP Biotin-Z Internalization Kit

Targeted conjugates are widely used to escort payloads to specific cell populations *in vitro* and *in vivo* for both basic research and pharmaceutical development. Desirable traits of a Targeting Agent include high specificity and rapid internalization. The Targeting Agent can be an antibody, peptide, protein, or any other molecule that recognizes a cell-surface marker.

The ZAP products allow a large number of targeting agents to be screened quickly and cost-efficiently for specificity, functional binding, internalization, and EC50 determination. The **NEW ZAP Biotin-Z Internalization Kits** are constructed using streptavidin (for use with biotinylated targeting agents), chemically attached to Saporin, the most potent of the plant ribosome-inactivating proteins.

ZAP products are combined with Targeting Candidates and collectively applied to plated cells. Once the materials have been administered, the targeting molecule directs the ZAP to the cells of interest, the complex is bound by the targeted cells, internalized, and the Saporin protein is released within the cytosol to inactivate the ribosomes, causing cell death. Cells not expressing the target are not affected.

The **NEW ZAP Biotin-Z Internalization Kit** contains all of the materials needed to screen your biotinylated targeting agent. Included in the kit are Streptavidin-ZAP, controls, and developing reagents for an *in vitro* assay. The user provides only the materials specific to their experiment (the biotinylated targeting agent, cells expressing the target, and culture reagents). An additional benefit of the biotin-streptavidin connection is that these conjugates can be used in an *in vivo* environment as well. For those customers who need biotin attached to a targeting candidate, ATS provides a biotinylation service. The biotinylated targeting agent will be returned to the customer with one of our Biotin-Z kits for no additional charge.

NEW! Beta-Testing Program

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 will have:

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

Beta Products have not been characterized or reported in scientific literature. This provides researchers with special Beta-pricing ($50 for 25 micrograms) 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.

Data submitted will be reviewed by the scientific team at ATS. If data is sufficient to prove specific activity of Beta material in either *in vivo* or *in vitro* conditions, the Beta Tester will be informed and product credit will be awarded to the first Beta Tester to publish.

Check the website for a current list of Beta-Test Products, and check back each quarter as new products are released in *Targeting Trends*.

Doug plays his clarinet and Kermit peeks out of the basket.

"Molecular Surgery for Scientists"

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Targeting Technology

Advanced Targeting Systems' technology - Molecular Neurosurgery - is a modification of one of the most widely used techniques: surgical lesioning of a region and observation of the effect.

Choose an ANTIBODY§ specific to your cell type.

SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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 the cover story**, one letter to each block, to solve the puzzle.

BALDROAR

NERVIETRAY

TWIRLOETER

NATIONSTARL

GIANTSNIFIC

Why the Zombie wanted to be a Neuroscientist.

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

ANSWER:

HE LOVED . . . !

WIN!

SOLVE the puzzle online with the correct solution by December 31, 2014.

WIN a 2015 ATS Calendar!

www.atsbio.com/news/14q4_teaser.html