Jan-Feb-Mar 2013
Volume 14, Issue 1

Targeting Trends

Reporting the latest news in Molecular Surgery

Role of cholinergic neurons in the nucleus accumbens and their involvement in schizophrenic pathology

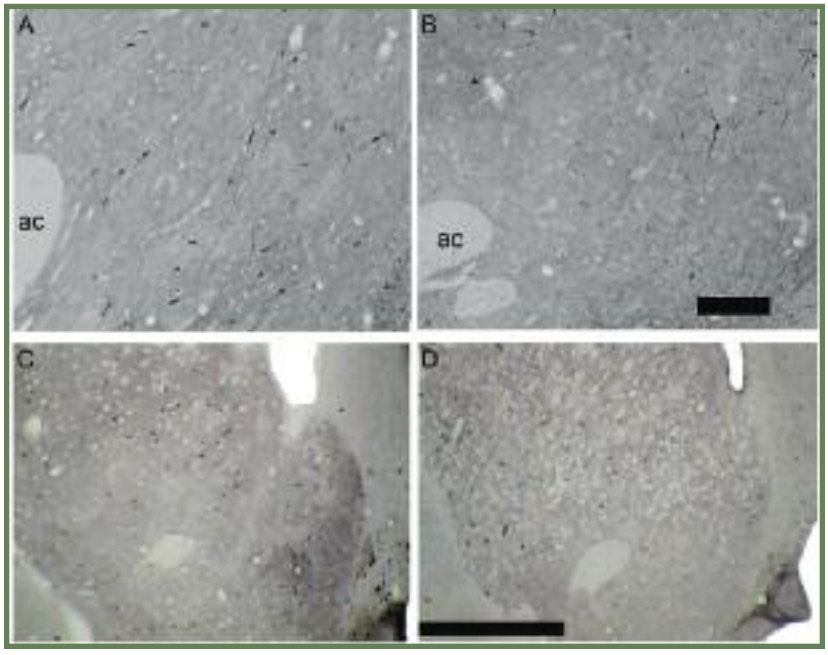
Contributed by François LaPlante. Dept of Psychiatry, McGill University, Montréal, QC, Canada

A post-mortem reduction in the density of cholinergic interneurons in the ventral striatum or nucleus accumbens (N.Acc.) has been reported in schizophrenic brains.[1,2] In this region the cholinergic interneurons interact anatomically and functionally with the dopaminergic nerve terminals notably to dampen the effects of excessive dopamine activity. We hypothesized that the lower level of cholinergic neurons and subsequently the acetylcholine release in the N.Acc may be relevant to the enhanced (ventral) striatal dopaminergic neurotransmission, well-described in schizophrenia, and may contribute to the emergence of schizophrenic symptoms.

The purpose of our work is to reproduce in rats the selective reduction of cholinergic interneurons in the N.Acc. and study the physiological and behavioral consequences of such lesions with relevance to schizophrenia. We employed the saporin immunotoxin targeting choline acetyltransferase (anti-ChAT-SAP; Cat. #IT-42), microinjected bilaterally (250-ng/side) into the N.Acc. of adult Sprague-Dawley rats. We found a localized and selective 40-50% loss of cholinergic interneurons (minimum two weeks post-lesion) with sparing of adjacent areas.[3] Similarly, the toxin infusion resulted in a 34% reduced tissue level of acetylcholine in the N.Acc.

We have previously shown that partial depletion of cholinergic neurons resulted in heightened behavioral sensitivity to amphetamine and impaired sensorimotor function[3] analogous to those seen in schizophrenia. Recently we observed that such lesions also significantly impaired performance in a T-maze task, a measurement of working memory. In addition, *in vivo* activation of dopamine release in the prefrontal cortex was markedly reduced; this deficit correlated

(continued on page 6)



Representative sections of ChAT-immunostained tissues of N.Acc. from rats that (A and C) received an intra-accumbens micro-injection of rabbit IgG-SAP (Cat. #IT-35; 250 ng) as control group, and (B and D) received an intra-accumbens micro-injection anti-ChAT-SAP (250 ng). The administration of anti-ChAT-SAP reduced significantly the amount of cholinergic interneurons at the injection site and spared the adjacent areas like the dorsal striatum. Scales A and B = 200 µm; C and D = 1 mm; ac: anterior commissure.

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Society for Neuroscience Poster of the Year Winner

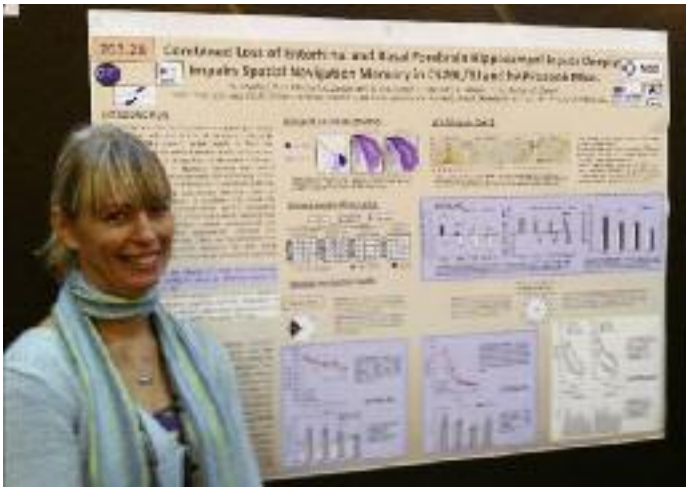
Doug Lappi congratulates Ko Zushida on the winning ATS Poster of the Year.

The Society for Neuroscience Meeting was a wonderful opportunity to meet scientists and see the research they've accomplished with our products. Each year, ATS chooses an Poster of the Year that is the culmination of a great idea, demonstrates targeted specificity and displays fantastic data. Congratulations this year to Ko Zushida and his colleagues.

ATS is already looking forward to next year. We hope to see *you* in San Diego, November 9-13, 2013.

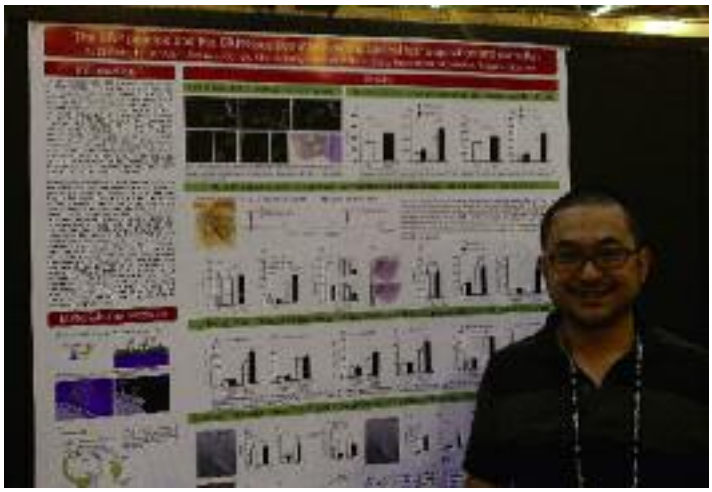
Honorable Mention went to Dr. Chantal Mathis who presented her poster ***"Combined loss of entorhinal and basal forebrain cholinergic hippocampal inputs deeply impairs spatial navigation memory in C57BL/6J and hAPPxapoE mice"*** authored by C. Mathis, P.-H. Moreau, C. Zerbinatt, R. Goutagny, B. Cosquer, K. Geiger, C. Kelche and J.C. Cassel. This poster showed, among many things, that human ApoE4 knocked-in mice treated with mu p75-SAP to eliminate cholinergic hippocampal neurons had greater learning deficits than ApoE3-knocked-in mice, consistent with the role of ApoE4 as a genetic risk factor for Alzheimer's Disease.

featuring mu p75-SAP (Cat. #IT-16)



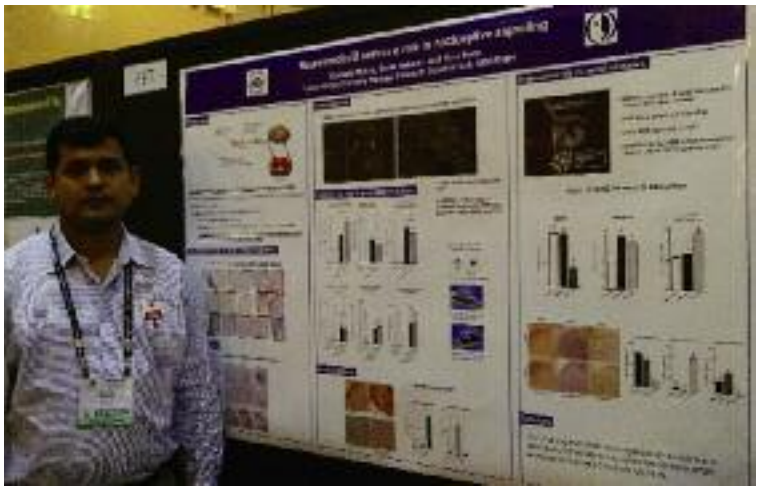
The Poster of the Year winner was Ko Zushida of Rutgers University who presented his poster ***"The GRP peptide and the GRPR-positive interneurons control fear acquisition and extinction"*** by K. Zushida, K. Light, S. Uchida, C. Hevi and G.P. Shumyatsky. This work involved the removal of GRPr-expressing inhibitory interneurons with Bombesin-SAP. Bombesin is the frog-skin analog of gastrin-releasing peptide. The result was enhancement of aspects of fear memory. The importance of fear acquisition and extinction has become a topic of major importance and these authors have found a nice tool for its examination.

featuring Bombesin-SAP (Cat. #IT-40)



Honorable Mention went to Santosh Mishra who presented his excellent poster from S.K. Mishra, S. Holzman and M.A. Hoon: ***"Neuromedin B serves a role in nociceptive signaling."*** They used NMB-SAP to specifically eliminate NMB receptor-expressing superficial dorsal horn interneurons and determined that they are required for responses to noxious heat, but not for reactions to mechanical and pruritic stimuli, indicating a role for these neurons in perception of thermal stimuli.

featuring NMB-SAP (custom conjugate)



Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Role for kisspeptin/neurokinin B/dynorphin (KNDy) neurons in cutaneous vasodilatation and the estrogen modulation of body temperature.

Mittelman-Smith MA, Williams H, Krajewski-Hall SJ, McMullen NT, Rance NE.
Proc Natl Acad Sci U S A 109(48):19846-19851, 2012.

Menopause is marked by estrogen withdrawal, and also by hot flushes. Given the fact that hypothalamic levels of kisspeptin/neurokinin B/dynorphin (KNDy) neurons are significantly altered in menopause, the authors investigated whether these neurons are involved in the generation of flushes. Rats received bilateral injections of NK3-SAP (Cat. #IT-63) into the arcuate nucleus – a total of 40 ng. Blank-SAP (Cat. #IT-21) was used as control. The data indicate that KNDy neurons promote cutaneous vasodilation, and play a role in 17β-estradiol modulation of body temperature, supporting the hypothesis that these neurons could play a role in the generation of hot flushes.

CD22 Antigen Is Broadly Expressed on Lung Cancer Cells and Is a Target for Antibody-Based Therapy.

Tuscano JM, Kato J, Pearson D, Xiong C, Newell L, Ma Y, Gandara DR, O'Donnell RT.
Cancer Res 72(21):5556-5565, 2012.

The median overall survival of patients with advanced, unresectable, non-small cell lung cancer is 9-12 mos. A potential therapeutic target is CD22, a protein expressed on lung cancer cells. The authors examined the use of the monoclonal antibody HB22.7 as an antitumor agent. To assess internalization of the antibody, it was first incubated with 10 µg/ml Mab-ZAP (Cat. #IT-04) then applied to two different cancer cell lines in culture. Analysis of cell viability demonstrated that CD22 internalized when bound by the antibody-toxin complex, suggesting that targeting CD22 has therapeutic potential.

Histamine release in the basal forebrain mediates cortical activation through cholinergic neurons.

Zant JC, Rozov S, Wigren HK, Panula P, Porkka-Heiskanen T.
J Neurosci 32(38):13244-13254, 2012.

The basal forebrain modulates many functions, among them the regulation of wakefulness and cortical arousal. Previous data has linked increases in histaminergic transmission to increases in wakefulness. In order to further investigate various facets of this system, the authors injected 230 ng of 192-IgG-SAP (Cat. #IT-01) into the horizontal diagonal band of Broca/substantia innominata/magnocellular preoptic area of rats. While control animals displayed several changes on administration of exogenous histamine, the lesioned animals had none of these changes.

Acetylcholine facilitates recovery of episodic memory after brain damage.

Croxson PL, Browning PG, Gaffan D, Baxter MG.
J Neurosci 32(40):13787-13795, 2012.

Episodic memory is controlled by several interconnected brain structures. The order in which these structures sustain damage can affect the processes lost. In this work the authors performed numerous bilateral injections of ME20.4-SAP (Cat. #IT-15) into the infero-temporal cortex, the medial surface of the temporal lobe, the perirhinal and entorhinal cortex, and the temporal pole of monkeys. These injections totaled 2.2-2.5 µg of conjugate. The results indicate that loss of cortical acetylcholine function will interfere with adaptation to memory impairments caused by structural damage in episodic memory centers.

Form and function of the M4 cell, an intrinsically photosensitive retinal ganglion cell type contributing to geniculocortical vision.

Estevez ME, Fogerson PM, Ilardi MC, Borghuis BG, Chan E, Weng S, Auferkorte ON, Demb JB, Berson DM.
J Neurosci 32(39):13608-13620, 2012.

Intrinsically photosensitive retinal ganglion cells (ipRGCs) are cells that contain the photopigment melanopsin. In this work the authors extensively characterize the M4 ipRGCs. A melanopsin antibody (Cat. #AB-N38) at a 1:10,000 dilution was used to determine the presence of melanopsin by immunohistochemistry.

Diffusion-weighted magnetic resonance imaging detection of basal forebrain cholinergic degeneration in a mouse model.

Kerbler GM, Hamlin AS, Pannek K, Kurniawan ND, Keller MD, Rose SE, Coulson EJ.
Neuroimage 66C:133-141, 2012.

The authors examined the effectiveness of diffusion MRI using diffusion tensor imaging (DTI) and probabilistic tractography in detecting cholinergic loss in a mouse model. Mice received bilateral 0.2-µg icv injections of mu p75-SAP (Cat. #IT-16). Rabbit IgG-SAP (Cat. #IT-35) was used as control. The animals were then examined using DTI. The data indicate that DTI is a valid technique for assessment of cholinergic loss in septo-hippocampal tracts as a result of Alzheimer's disease.

(continued on page 4)

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

Targeting Topics: Recent Scientific References

(continued from page 3)

Photochemical internalization (PCI) of HER2-targeted toxins: Synergy is dependent on the treatment sequence.

Berstad MB, Weyergang A, Berg K.
Biochim Biophys Acta 1820(12):1849-1858, 2012.

A majority of patients develop acquired resistance to trastuzumab, the monoclonal antibody recognizing HER2, coupled to a toxin as a breast cancer therapeutic. One of the modes of resistance is that the therapeutic molecule is trapped inside an endocytic vesicle. PCI is a technique that facilitates cytosolic release of molecules in vesicles. The authors investigated the potency of biotinylated trastuzumab combined with streptavidin-ZAP (Cat. #IT-27) on several cell lines.

The effect of the steroid sulfatase inhibitor (p-O-sulfamoyl)-tetradecanoyl tyramine (DU-14) on learning and memory in rats with selective lesion of septal-hippocampal cholinergic tract.

Babalola PA, Fitz NF, Gibbs RB, Flaherty PT, Li PK, Johnson DA.
Neurobiol Learn Mem 98(3):303-310, 2012.

Steroid sulfatase inhibitors such as dehydroepiandrosterone (DHEAS) have memory-enhancing effects. Working with both DHEAS and the steroid sulfatase inhibitor DU-14, the authors examined cholinergic function by infusing 0.2 µg of 192-IgG-SAP (Cat. #IT-01) into the medial septum of rats. The results indicate that memory associated with contextual fear is facilitated by steroid sulfatase inhibition, but acquisition of spatial memory is impaired by these same lesions.

Cholinergic degeneration is associated with increased plaque deposition and cognitive impairment in APPswe/PS1dE9 mice.

Laursen B, Mork A, Plath N, Kristiansen U, Bastlund JF.
Behav Brain Res Epub 2012.

Extracellular plaques containing amyloid β-peptides (Aβ) and cholinergic dysfunction are two of the main hallmarks of Alzheimer's disease. Using a transgenic mouse line that displays an age-related increase in plaque deposition, the authors examined the relationship between cholinergic degeneration and Aβ overexpresssion. Mice received 0.9-µg bilateral icv injections of mu p75-SAP (Cat. #IT-16). Working memory was significantly impaired in lesioned mice with plaques, and the plaque burden was increased as compared to wild-type mice that also received a lesion.

IB4(+) nociceptors mediate persistent muscle pain induced by GDNF.

Alvarez P, Chen X, Bogen O, Green PG, Levine JD.
J Neurophysiol 108(9):2545-2553, 2012.

GDNF is found in skeletal muscle and can trigger mechanical hyperalgesia. The authors administered a 3.2-µg intrathecal dose of IB4-SAP (Cat. #IT-10) to rats. Loss of the IB4(+) nociceptors led to decreased hyperalgesic priming as well as a reduction in GDNF-induced hyperalgesia. These data indicate that GDNF plays a role in mediating induction of pain.

Efficacy of a CD22-targeted antibody-saporin conjugate in a xenograft model of precursor-B cell acute lymphoblastic leukemia.

Kato J, Satake N, O'Donnell RT, Abuhay M, Lewis C, Tuscano JM.
Leuk Res Epub 2012.

Most cases of acute lymphoblastic leukemia (ALL) are of B-cell lineage. Although children with ALL have a high survival rate, there is a subset of children with a much lower survival rate, and long-term side effects from treatment are problematic. CD22 has been suggested as a therapeutic target because it is not present on hematopoietic stem cells, therefore allowing regeneration of normal B cells following depletion of malignant B cells. The authors used a custom conjugate of the antibody HB22.7 and saporin to demonstrate specific toxicity against pre-B ALL cell lines. Mouse IgG-SAP (Cat. #IT-18) was used as a control.

Insights into the mechanism of cell death induced by saporin delivered into cancer cells by an antibody fusion protein targeting the transferrin receptor 1.

Daniels-Wells TR, Helguera G, Rodriguez JA, Leoh LS, Erb MA, Diamante G, Casero D, Matteo P, Martinez-Maza O, Penichet ML.
Toxicol In Vitro Epub 2012.

The antibody-avidin fusion protein ch128.1Av has been shown to target the

(continued on page 6)

Spring Brain Conference
March 20-23, 2013 • Sedona, AZ

AACR • April 4-10, 2013
Washington, DC

Upcoming Events

Spring Brain Conference

The 24th annual Spring Brain Conference is March 20-23 in Sedona, Arizona. On the meeting agenda are seven plenary sessions, an informal poster session, and you won't want to miss the keynote speakers. Their session information is listed here. A full program schedule is on the website at www.SpringBrain.org.

SBC is not just for science. Sedona is a beautiful destination that will appeal to scientists and non-scientists, plus attendees young and old. Art galleries abound. There are opportunites for tours or self-guided experiences among the famous red rocks. Two golf tournaments will be held at the highly acclaimed Seven Canyons course. More information is on www.SpringBrain.org

Wednesday Keynote Speaker

Seymour Reichlin, MD, PhD,
Tufts Univ School of Medicine

Did Goliath Have a Growth Hormone Secreting Pituitary Adenoma Compressing His Optic Nerves? (And What Does That Have to Do with the Birth of Neuroendocrinology?)

This speaker will be kicking off SBC with a history of neuroendocrinology. Don't be lulled by the topic. Seymour Reichlin will be keeping everyone entertained and hanging on every word.

Thursday Keynote Speaker

Hans S Keirstead, Professor, University of California at Irvine

Stem Cell-Based Approaches to Treat Spinal Cord Injury

Human cell-based therapies require well-defined, high purity populations of cells, for both regulatory and ethical reasons. We have generated FDA-compliant oligodendrocyte progenitor cells from human embryonic stem cells suitable for addressing clinical indications characterized by oligodendrocyte loss, and developed the technology to address pre-clinical efficacy and pre-clinical safety concerns; this technology was adopted and further developed by Geron Corporation and is in clinical testing in the USA. More recently, we have generated a clinical grade product of motor neuron progenitors derived from human embryonic stem cells suitable for addressing indications characterized by lower motor neuron loss. This cell population has been made in a clinically compliant manner, and tested for safety and efficacy. Recent interactions with the US FDA and UK MHRA indicate that the program will soon be approved for testing in humans. We have also generated a clinical grade product of neuronal progenitors derived from human embryonic stem cells suitable for addressing indications characterized by neuron loss. We have developed a clinically-compliant method of inhibiting PTEN within these neuronal progenitors, which increases axonal extension rate, a desired property for spinal cord regeneration. Lastly, we have developed a method of overexpressing a cocktail of transcription factors in mature astrocytes reverts them to an embryonic state. We hypothesize that this process restores their pro-regenerative embryonic properties and thus permits axonal regeneration by re-establishing the pro-regenerative environment characteristic of embryonic animals.

Denise Higgins at Crescent Moon Day Use Area at Red Rock Crossing

Friday Keynote Speaker

Mark Baxter, Professor,
Mount Sinai School of Medicine

Animal models of cognitive impairment after general anesthesia: From the grave to the cradle

It has become appreciated that exposure to general anesthesia can have lasting effects on brain function long after recovery from the state of anesthesia. This includes post-operative cognitive dysfunction (POCD) in elderly individuals, and anesthetic neurotoxicity early in development. Animal models have been critical complements to human clinical studies in understanding these phenomena and providing potential solutions, so that unintended long-term effects of anesthetic exposure can be minimized or avoided.

Executive Golf Course at Poco Diablo Resort as seen from guest room.

Register today at www.SpringBrain.org

Role of cholinergic neurons in the nucleus accumbens and their involvement in schizophrenic pathology

(continued from page 1)

significantly with cognitive impairments.[4] These data suggest that intra-accumbens lesions of the cholinergic neurons trigger not only a local hyper-responsiveness to dopamine but also widespread functional impairments in prefrontal cortical dopamine functions similarly as proposed in the pathology of schizophrenia. Studies to describe mechanistic consequences of cholinergic lesions on dopamine neurotransmission are in progress. Additionally, as ChAT spends time on the membrane, it can be used to target cholinergic neurons.[5]

References

1. Holt DJ, Herman MM, Hyde TM, Kleinman JE, Sinton CM, German DC, Hersh LB, Graybiel AM, Saper CB. 1999. Evidence for a deficit in cholinergic interneurons in the striatum in schizophrenia. *Neuroscience* 94:21-31.
2. Holt DJ, Bachus SE, Hyde TM, Wittie M, Herman MM, Vangel M, Saper CB, Kleinman JE. 2005. Reduced density of cholinergic interneurons in the ventral striatum in schizophrenia: an in situ hybridization study. *Biol Psychiatry* 58:408-416.
3. Laplante F, Lappi DA, Sullivan RM. 2011. Cholinergic depletion in the nucleus accumbens: Effects on amphetamine response and sensorimotor gating. *Prog Neuropsychopharmacol Biol Psychiatry* 35:501-509.
4. Laplante F, Zhang ZW, Huppe-Gourgues F, Dufresne MM, Vaucher E, Sullivan RM. 2012. Cholinergic depletion in nucleus accumbens impairs mesocortical dopamine activation and cognitive function in rats. *Neuropharmacology* 63:1075-1084.
5. Badamchian M and Carroll PT. 1985. Molecular weight determinations of soluble and membrane-bound fractions of choline O-acetyltransferase in rat brain. *J Neurosci* 5(8):1955-1964.

Targeting Teaser Winners

The solution to the puzzle was: Jumbles: ROBUSTLY
QUIZ
DEMENTIA
COGNITIVE
INSULT

What motivated the scientist to push forward in his research.

Answer: A... BURNING QUESTION!

Congratulations to the puzzle solvers from last quarter. Each winner has received $100 credit towards research product purchases from Advanced Targeting Systems.

Solve this quarter's Teaser online at: www.ATSbio.com/news/13q1_teaser.html

Targeting Topics: Recent Scientific References

(continued from page 4)

human transferrin receptor 1 (TfR1) and kill malignant B cells by blocking the use of iron. Combination of this construct with a mono-biotinylated saporin custom conjugate produces an iron-independent toxicity to TfR1-expressing cells, even those that are resistant to ch128.1Av alone. The saporin-containing conjugate induces a transcriptional response consistent with oxidative stress and DNA damage. The data also show that the saporin conjugate is not toxic to human hematopoeietic stem cells.

Identification and characterization of a sleep-active cell group in the rostral medullary brainstem.

Anaclet C, Lin JS, Vetrivelan R, Krenzer M, Vong L, Fuller PM, Lu J.

J Neurosci 32(50):17970-17976, 2012.

The authors attempt to locate and identify specific neuronal populations that promote sleep. One method utilized was 130-330 pg injections of orexin-SAP* into the parafacial zone. These results establish the parafacial zone as a delimited node of sleep-active neurons.

Metabolic effects of chronic sleep restriction in rats.

Vetrivelan R, Fuller PM, Yokota S, Lu J, Saper CB.

Sleep 35(11):1511-1520, 2012.

In order to investigate whether there is a correlation between sleep and weight the authors administered 200 nl of a 0.1% solution of orexin-SAP* to the ventrolateral preoptic area of rats. Although the lesioned animals slept less than the controls, weight gain was slower than controls.

**Note: A new version of Orexin-SAP is in production. Contact ATS if you are interesting in testing it.*

Targeting Tools: Featured Products

Anti-ChAT-SAP

Choline acetyltransferase (ChAT) catalyzes the synthesis of the neurotransmitter acetylcholine (ACh) from choline and acetyl-CoA in cholinergic neurons. ChAT serves as a specific marker for cholinergic neurons in both peripheral and central nervous systems. Dysfuntion of cholinergic neurons underlies aspects of clinical symptoms found in neurological and psychiatric disorders such as Alzheimer's disease, Down and Rett syndromes.

Evidence shows that the enzyme choline-O-acetyltransferase (ChAT) exists in two forms inside cholinergic nerve terminals, a soluble hydrophilic form and the membrane-associated amphiphilic form. As an example, membrane-bound ChAT was shown to be localized as a peripheral protein that is attached to synaptosomal plasma membrane (Gabrielle *et al*. 2003). Membrane-bound ChAT has served as the feature condition that allows Advanced Targeting Systems to apply their technology through an affinity-purified antibody to ChAT conjugated to saporin, the ribosome-inactivating protein, to specifically target and eliminate those specific cells.

Anti-ChAT-SAP is made with an antibody using a 22-amino acid peptide from porcine ChAT (GLF SSY RLP GHT QDT LVA QKSS). The targeted toxin recognizes porcine ChAT and is expected to cross-react with rat, mouse, and human.

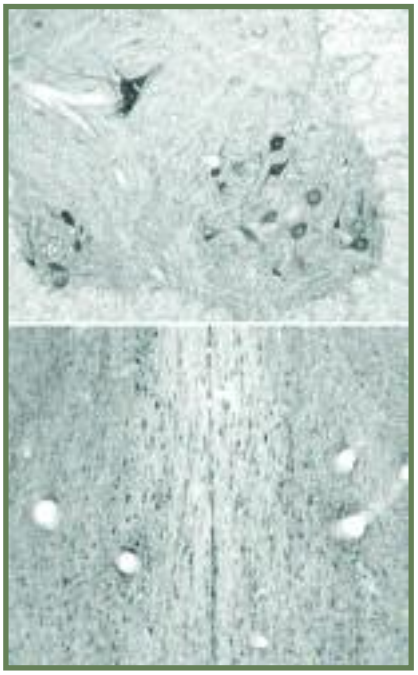
Gabrielle P, Jeana M, Lorenza EC (2003) Cytosolic choline acetyltransferase binds specifically to cholinergic plasma membrane of rat brain synaptosomes to generate membrane-bound enzyme. *Neurochem Res* 28(3-4):543-549.

Targeted Toxins

Anti-ChAT-SAP eliminates cells expressing ChAT
available individually (IT-42)
available as a kit (KIT-42) including
Saporin (PR-01), Rabbit IgG-SAP (IT-35) and Anti-ChAT affinity-purified (AB-N34AP)

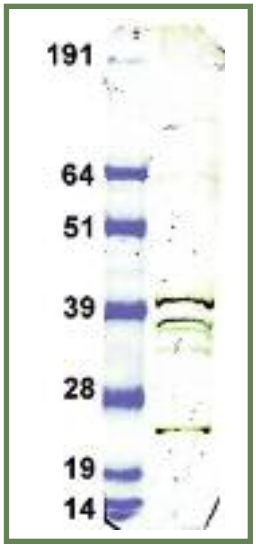
Other Conjugates Targeting the Cholinergic System
192-IgG-SAP (IT-01)
eliminates cells expressing $p75^{NTR}$ in rat
ME20.4-SAP (IT-15)
eliminates cells expressing $p75^{NTR}$ in mammals
mu p75-SAP (IT-16)
eliminates cells expressing $p75^{NTR}$ in mouse

visit www.ATSbio.com for a complete list of products



A fixed section of rat spinal cord (A) and forebrain (B) was stained with our anti-ChAT (Cat. #AB-N34; 1:2000-4000 dilution).
Courtesy of Dr. Ronald G. Wiley. and Robert Kline at Vanderbilt University, Nashville, TN.

Anti-ChAT affinity-purified (Cat. #AB-N34AP)
Lane 1: Crude rat brain whole cell extract probed with at 1:100 dilution
Lane 2: Molecular weight standards (Invitrogen SeeBlue).



Antibodies

Antibody to ChAT (AB-N34)
affinity-purified (AB-N34AP)
biotin-labeled affinity-purified (BT-N34AP)
Alexa488-labeled affinity-purified (FL-N34AP)

Gangsta enjoys the last rays of the afternoon sunshine.

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

10451 Roselle Street #300
San Diego, CA 92121

PRESORTED STD.
U.S. POSTAGE
PAID
SAN DIEGO, CA
PERMIST # 2686

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.

INLOCHE

ZEPHORICHINAS

NUTCIFON

SNORETINNEUR

FUNNISOI

How the intern thought a future of science would impact her future.

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

ANSWER: SHE WOULD LIVE A . . . ○○○○ ○○ ○○ .

WIN $100.00

Limit one entry per laboratory. Credits expire after one year.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by March 15, 2013.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

Please correct the address information above and provide the following:

Your Name:
Phone:
Fax:
Email:

Apr-May-Jun 2013
Volume 14, Issue 2

Targeting Trends

Reporting the latest news in Molecular Surgery

Antibodies to glycosphingolipids: An attractive tool for targeted delivery of cytotoxic agents to tumor cells

Contributed by Jose Luis Daniotti, Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC, UNC-CONICET), Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

Glycosphingolipids (GSLs) are amphipathic molecules consisting of a ceramide lipid moiety linked to a glycan chain of variable length and structure. Among these are found the gangliosides, which are mono- or multi-sialosylated GSLs mainly located in the outer layer of the plasma membrane of vertebrate cells. These are expressed in cell type- and developmental-specific patterns and are major components of nerve cells, being implicated in many physiological processes, including growth, differentiation, migration and apoptosis. Furthermore, gangliosides have been associated with a wide range of pathological processes, as they are receptors for both viruses and antibodies.[1]

The biosynthesis of gangliosides starts at the endoplasmic reticulum and continues through a combination of glycosyltransferase activities at the Golgi complex, which is followed by vesicular delivery to the plasma membrane.[1-3] Recently, our laboratory and other researchers have demonstrated that the regulation of GSL expression also occurs at the cell surface, where both glycosyltransferases and glycohydrolases locally modulate the cellular glycolipid pattern.[4-6]

Over recent years, we have paid particular attention to the synthesis and function of ganglioside GD3. Although this disialo glycolipid is highly expressed at the early developmental stages of the central nerve system, at later developmental stages the GD3 content declines and other gangliosides become major players.[1,3] Despite the expression levels of gangliosides in general, and of GD3 in particular, being very low and restricted to adult extra neural tissues, GD3 is highly expressed in tumor cells, especially melanomas. It is also overexpressed in neuroectodermal tumors (neuroblastoma and glioma) and carcinomas of lung, breast, colon, prostate and ovary.[7] For these reasons, ganglioside GD3 has received considerable attention as a promising immunotherapeutic target for cancer therapy. As such, it has been used for passive and active immunotherapy of

(continued on page 6)

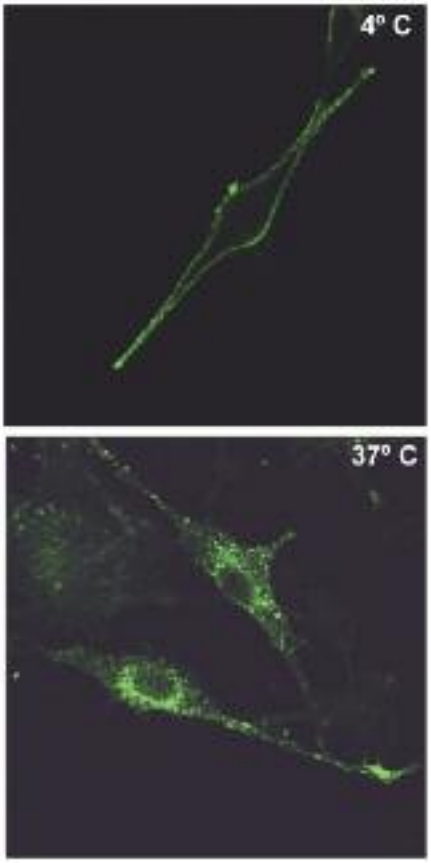


Figure 1. Internalization of antibody to GD3 (R24) in human SK-Mel-28 melanoma cells. Human SK-Mel-28 cells, previously characterized as expressing endogenous GD3, were incubated with antibody to GD3 for 45 min at 4ºC (top panel). Then, the temperature was shifted to 37 ºC to allow endocytosis of the complex GD3-R24 and cells were fixed at 30 min (bottom panel). R24 antibody was detected by using anti-mouse IgG conjugated with Alexa488. Single confocal sections of 0.8 µm were taken parallel to the coverslip. Modified from Ref. 9.

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

2013 Spring Brain Conference Recap

March 20-23rd, ATS sponsored the Spring Brain Conference in beautiful Sedona, Arizona. The weather was fabulous, the red rocks were as great as they have been for the last 300,000,000 years and the science was top notch, with excellent Neuroscience researchers coming from all over the US (and from South Korea, too) to exchange ideas and to see what's new in the Neurosciences.

After an introductory dinner Wednesday evening, Thursday started with one of the most controversial and important issues facing the US. **Session 1: Autism**. Gene Blatt and Tom Kemper (Boston Univ) discussed facets of the Harvard Brain Tissue Resource Center at McLean Hospital and the histology and chemistry one can find in looking through the brains of autistics. Contrary to common belief, there are significant documented changes. Judy van de Water (UC Davis) discussed aspects of auto-immunity and Isaac Pessah (UC Davis) gave a correlation between environmental factors and autism that could very well explain the increase in diagnoses.

Session 2: Traumatic Brain Injury, Dave Hovda (UCLA) and Jonathon Lifshitz (Phoenix Children's Hospital) spoke about metabolic changes after trauma and brain vulnerability. Lee Shapiro (Texas A&M) discussed a new, more precise animal model for TBI and Eldon Geisert (Univ Tennessee) spoke about the results of plugging information from optic nerve crush into his GeneNetwork.org algorithms to know and understand network connectivity.

On Thursday evening, our keynote speaker was **Seymour Reichlin MD, PhD, Professor Emeritus at Tufts** who related a fascinating description of the dramas that occurred in the establishment of **Neuroendocrinology**—it's quite a story. Among the dramas were the struggle for the Nobel Prize, the sometimes bitter competition between the key players and the magic of characterizing the connection and control of the mind and the body, as happens between the hypothalamus and the pituitary.

Friday, **Session 3: Pain** started with Frank Porreca (Univ Arizona) and Narrender Gavva (Amgen) speaking of the difficulties of coming up with new pain remedies. Frank emphasized that the animal models of the last 20 years were not producing anything new and that the reflex models should be replaced by models more suited to the pain situation. Dr Gavva gave a great analysis of the challenges that industry has to develop new medicines and recommended prioritizing human genetics targets with big effect size and pursuing combinations into a single pill.

Next, **Session 4: Gambling, Sex and Drugs** (topics certainly of some interest) focused on animal models of what makes us do those things. Celeste Napier (Rush Univ) spoke on the changes resulting in obsessive behavior that happen in Parkinson's Disease. Kathy Cunningham (Texas MC Galveston) discussed an imbalance in serotonin receptors (rather than transporter) in impulsive activity and Lique Coolen (Univ Mississippi) spoke about what happens when methamphetamines mix with sex (not a great pairing).

Spring Brain Conference

www.SpringBrain.org

Ending the morning were speakers from Washington State **Session 5: Hindbrain**. Sue Ritter spoke of glucose-sensing mechanisms and neural circuitry utilized by hindbrain catecholamine neurons (with projections into the spinal cord). James Peters talked about vagal afferent synapses, and TRP channels in the synaptic function of vagal neurons. Bob Ritter spoke about vagal afferent signaling by NMDA-type glutamate receptors allowing feeding control.

Friday night the **keynote speaker** was **Mark Baxter from Mt. Sinai MC** who spoke about **Cognition after Anesthesia**. This has been known to be at times a devastating problem in the elderly, but Mark presented data that there can be serious problems from anesthesia in the very young which can cause serious learning issues.

Saturday morning began with **Session 6: Breathing and its Control.** Jack Feldman (UCLA) talked about the rhythm and coordination needed for everyday breathing, the influence of blocking GABA and glycine receptors on the vagus nerve, and the role the preBötzinger complex (and Botzinger complex) plays in normal respiratory patterns. Gordon Mitchell (Univ Wisconsin) presented the importance of retraining respiration after spinal cord injury (asphyxiation being a major cause of death there). Ralph Fregosi (Univ Arizona) spoke about the effects of nicotine in the young. George Richerson (Univ Iowa) presented material on epileptic seizure and sudden death in epileptics due to respiratory failure.

Session 7: Cell-Type Specific Circuits showed new methods in cell tracing in the Neurosciences. Xiangmin Xu (UC Irvine) showed the trans-synaptic powers of the rabies virus for detailing long-range neuronal connectivity. David Lyon (UC Irvine) then extended the discussion, speaking on the visual cortex processes. Steve van Hooser (Brandeis) talked about 2-photon imaging and viral vectors in early development of direction selective circuits in ferret visual cortex. These were beautiful and impressive demonstrations of the techniques that will become important in the Brain Activity Map Project.

This was a great conference that allows researchers to pick up new ideas on how to improve their research and make collaborations. There is also time to talk, which isn't always found in other Neuroscience meetings.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Direct Retino-Raphe Projection Alters Serotonergic Tone and Affective Behavior.

Ren C, Luan L, Wui-Man Lau B, *et al.*
Neuropsychopharmacology Epub2013.

Although recent work has shown that some intrinsically photosensitive retinal ganglion cells (ipRGCs) are responsible for processing nonimage-forming visual functions, it is unclear whether the ipRGCs or conventional RGCs modulate affective behavior. The authors injected 2 µg of melanopsin-SAP (Cat. #IT-44) into each eye of rats, or biotinylated CTB monoclonal antibody coupled to Streptavidin-ZAP (Cat. #IT-27). The data suggest that retino-raphe signals modulate dorsal raphe nucleus serotonergic tone and affective behavior.

Stable Respiratory Activity Requires Both P/Q-Type and N-Type Voltage-Gated Calcium Channels.

Koch H, Zanella S, Elsen GE, *et al.*
J Neurosci 33(8):3633-3645, 2013.

Pharmacological experiments have suggested that sighs and normal respiration are highly dependent on calcium currents carried by P/Q channels. Using transgenic mice missing the Cav2.1 pore-forming α1A subunit the authors demonstrate that loss of P/Q-type calcium channels results in compromised breathing, sighing, neuromodulation, and leads to early death. A neurokinin-1 receptor antibody (Cat. #AB-33AP) at a 1:500 dilution was used for immunohistochemistry in this work.

Single domain antibodies for the detection of ricin using silicon photonic microring resonator arrays.

Shia WW, Bailey RC.
Anal Chem 85(2):805-810, 2013.

A major hurdle to clear in the fight against bioterrorism is the ability to identify various biowarfare agents. One of the more difficult substances to identify is ricin. This work describes the use of single domain antibodies to identify ricin in a microring resonator array assay. Saporin (Cat. #PR-01) along with affinity purified chicken anti-saporin (Cat. #AB-17AP) were used as controls when constructing the assay. The results demonstrate the feasibility of using microring resonator arrays for the detection of biowarfare agents.

Morphine hyperalgesia gated through microglia-mediated disruption of neuronal Cl^- homeostasis.

Ferrini F, Trang T, Mattioli TA, *et al.*
Nat Neurosci. 2013 Feb;16(2):183-92.

Although morphine is the drug of choice in dealing with chronic pain, it paradoxically can produce a hyperalgesic state. The authors examined the issue from several different angles. One method was to eliminate spinal microglia of rats through the intrathecal application of 16-32 µg of Mac-1-SAP (Cat. #IT-33). 20 µg of saporin (Cat. #PR-01) was used as a control. It was found that P2X4 receptors expressed by microglia were necessary for the development of morphine hyperalgesia, but not morphine tolerance.

Partial loss in septo-hippocampal cholinergic neurons alters memory-dependent measures of brain connectivity without overt memory deficits.

Brayda-Bruno L, Mons N, Yee BK, Micheau J, Abrous DN, Nogues X, Marighetto A.
Neurobiol Dis Epub2013.

The authors examined whether partial degeneration of septo-hippocampal neurons alters brain activity patterns even without overt memory loss. Mice received 45 ng of mu p75-SAP (Cat. #IT-16) into the medial septal area. Lesioned animals had significantly altered functional activities in the brain, despite lack of an overt behavioral deficit. Some changes observed are also altered with the initial signs of Alzheimer's disease.

Lesions of the basal forebrain cholinergic system in mice disrupt idiothetic navigation.

Hamlin AS, Windels F, Boskovic Z, Sah P, Coulson EJ.
PLoS One 8(1):e53472, 2013.

Alzheimer's disease patients perform poorly on spatial navigation tests requiring either distal cues (allothetic) or body-centered cues (idiothetic). The authors used 0.2 µg bilateral infusions of mu p75-SAP (Cat. #IT-16) into the lateral ventricles of mice to examine the hypothesis that the cholinergic medial septo-hippocampal circuit is important for idiothetic navigation. Rabbit IgG-SAP (Cat. #IT-35) was used as a control. Lesioned animals were similar to controls in contextual fear conditioning, spatial working memory, as well as several other parameters. But exploratory behavior requiring idiothetic signals was very disorganized, indicating that cholinergic cells are vital to idiothetic navigation.

Rapid beta-Amyloid Deposition and Cognitive Impairment After Cholinergic Denervation in APP/PS1 Mice.

Ramos-Rodriguez JJ, Pacheco-Herrero M, Thyssen D, *et al.*
J Neuropathol Exp Neurol Epub2013.

The authors investigated whether specific cholinergic neurodegeneration is responsible for the deposition of plaques. APPswe/PS1dE9 transgenic mice received bilateral icv injections of 1-1.2 µg of mu p75-SAP (Cat. #IT-16) into the basal forebrain. Although the transgenic mice show plaque deposition, they do not exhibit other signs of Alzheimer's disease. Lesioned transgenic animals, however, displayed increased β-amyloid plaque deposition, increased Tau phosphorylation, and early memory impairment that worsened with age.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Targeted Delivery of Immunotoxin by Antibody to Ganglioside GD3: A Novel Drug Delivery Route for Tumor Cells.

Torres Demichelis V, Vilcaes AA, Iglesias-Bartolome R, Ruggiero FM, Daniotti JL.
PLoS One 8(1):e55304, 2013.

The authors used the mouse monoclonal antibody R24 against ganglioside G3 with Mab-ZAP (Cat. #IT-04) to test the viability of ganglioside G3 as a cancer therapy target. Varying concentrations of R24 were used on various cell lines with either 0.95 nM or 9.5 nM Mab-ZAP depending on the cell line. *(See cover article for more information.)*

Yohimbine anxiogenesis in the elevated plus maze requires hindbrain noradrenergic neurons that target the anterior ventrolateral bed nucleus of the stria terminalis.

Zheng H, Rinaman L.
Eur J Neurosci Epub2013.

The anterior ventrolateral bed nucleus of the stria terminalis (vIBST) appears to be important for increased noradrenergic signaling to trigger anxiety-like behavior. 42.8 ng of anti-DBH-SAP (Cat. #IT-03) was administered to the vIBST of rats in bilateral injections. Elimination of noradrenergic neurons in the vIBST abolished yohimbine-induced anxiogenesis in an elevated plus maze, indicating that hindbrain noradrenergic neurons targeting the vIBST are involved in this mechanism.

Long-term effects of selective immunolesions of cholinergic neurons of the nucleus basalis magnocellularis on the ascending cholinergic pathways in the rat: A model for Alzheimer's disease.

Szigeti C, Bencsik N, Simonka AJ, *et al.*
Brain Res Bull 94C:9-16, 2013.

192-IgG-SAP (Cat. #IT-01) has been used extensively to generate models of Alzheimer's disease in rats. In this work, the authors detailed the time course of neuronal loss with an eye on potential recovery from the lesion. The nucleus basalis magnocellularis of rats was

injected with 75 ng of 192-IgG-SAP (Cat. #IT-01) and long-term changes were tracked by immunohistochemistry. While some acetylcholinesterase neurons, considered cholinoceptive, were lost, choline acetyltransferase (cholinergic) neurons sustained a massive irreversible reduction in number.

Reduction in cholinergic interneuron density in the nucleus accumbens attenuates local extracellular dopamine release in response to stress or amphetamine.

Laplante F, Dufresne MM, Ouboudinar J, Ochoa-Sanchez R, Sullivan RM.
Synapse 67(1):21-29, 2013.

The authors examined whether excessive dopamine neurotransmission in the mesolimbic system is due to higher levels of presynaptic or postsynaptic dopamine. Rats received 250-ng bilateral injections of anti-ChAT-SAP (Cat. #IT-42) into the nucleus accumbens. Rabbit IgG-SAP (Cat. #IT-35) was used as a control. The data suggest that reduction of cholinergic interneurons in the nucleus accumbens suppresses presynaptic dopamine release. *(Also see cover article for Q1 2013.)*

Collateral damage and compensatory changes after injection of a toxin targeting neurons with the neurokinin-1 receptor in the nucleus tractus solitarii of rat.

Lin LH, Nitschke Dragon D, Talman WT.
J Chem Neuroanat 43(2):141-148, 2012.

The authors administered 9 ng of SSP-SAP (Cat. #IT-11) into the NTS and looked for differences in expression of neuronal markers.

Efficacy and toxicity of a CD22-targeted antibody-saporin conjugate in a xenograft model of non-Hodgkin's lymphoma.

Kato J, O'Donnell RT, Abuhay M, Tuscano JM.
Oncoimmunology 1(9):1469-1475, 2012.

In this work, the authors use a custom conjugate of anti-CD22 (mAb HB22.7) and saporin in a cytotoxicity assay on non-Hodgkin's lymphoma cell lines, as well as in a mouse tumor model. The dosing for the tumor model was 1 mg conjugate per kg of animal. Mouse IgG-SAP (Cat. #IT-18) was used as a control.

Sudden death following selective neuronal lesions in the rat nucleus tractus solitarii.

Talman WT, Lin LH.
Auton Neurosci Epub2012.

The authors used SSP-SAP (Cat. #IT-11) and anti-DBH-SAP (Cat. #IT-03) to examine the role of NK-1r-expressing neurons and catecholaminergic neurons in baroreflex control in the NTS. Either 3 ng of SSP-SAP or 42 ng of anti-DBH-SAP was injected into the NTS of rats and baroreflex function was compared 7 days later.

Intraneural OX7-saporin for neuroma-in-continuity in a rat model.

Mavrogenis AF, Pavlakis K, Stamatoukou A, *et al.*
Eur J Orthop Surg Traumatol Epub2012.

In 19 of the 24 OX7-SAP (2 µl; Cat. #IT-02) specimens, histology showed inhibition of neuroma-in-continuity formation.

Physiology of the orexinergic/hypocretinergic system: a revisit in 2012.

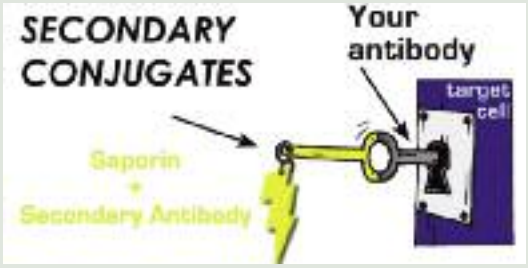
Kukkonen JP.
Am J Physiol Cell Physiol 304(1):C2-32, 2013.

This review updates an original review from a decade ago on the subject of orexins.

**Note: A new version of Orexin-SAP is in production. Contact ATS if you are interested in testing it.*

Targeting Talk: Product Questions

Q: How can I target B-cells? Which secondary conjugate should I use?



A: ATS makes secondary conjugates that use your primary antibody to target cells for elimination. Just mix your primary antibody with a secondary antibody conjugated to the ribosome-inactivating protein, Saporin, to screen your antibodies. The cells targeted by your primary antibody are eliminated.

Hum-ZAP is made with a bivalent secondary antibody that recognizes whole IgG. B-Cells have endogenous IgGs.

Fab-ZAP is made with a monovalent secondary antibody that recognizes whole IgG. B-Cells have endogenous IgGs.

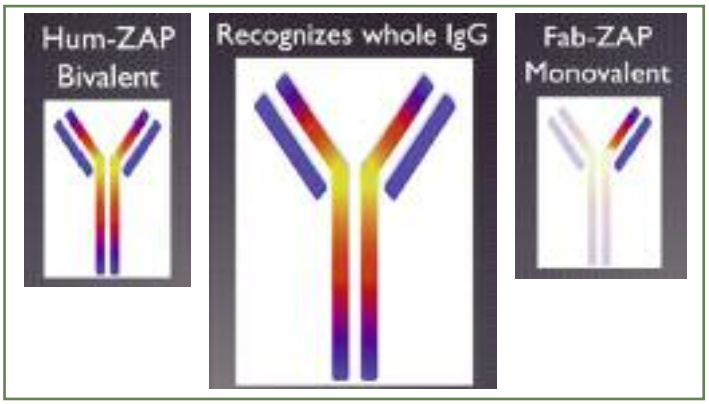
FabFc-ZAP is made with a monovalent secondary antibody that recognizes ONLY the FC portion of IgG. **FabFc-ZAP is an excellent choice!**

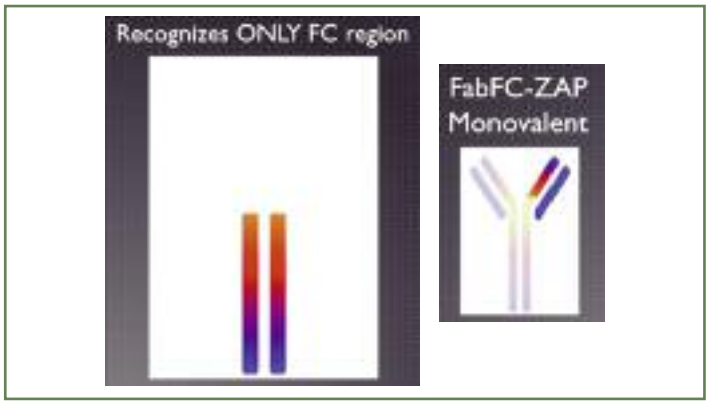
Q&A Products

Hum-ZAP (IT-22)

Fab-ZAP (IT-48, IT-51, IT-55, IT-57)

FabFc-ZAP (IT-65, IT-66)





Q&A Product

FGF-SAP (IT-38)

Q: Can you comment on the mechanism by which the SAP toxin is cleaved off from the antibody? Your website indicates that the cleavage occurs in the endosome. I just want to verify that it is not cleaved in the lysosome.

A: From what we have gathered, there is a great probability that something gets broken in the endosome. We know this from the peptide ligand toxins that bind to GPCRs. They would be rapidly returned to the cell surface through receptor recycling if there wasn't some sort of cleavage. In the case of FGF-SAP, e.g., we know that FGF is extensively degraded in the endosome through proteolytic degradation (Lappi *et al*, 1994). There is occasionally a disulfide linker between the toxin and antibody, but there is some controversy that this is cleaved: many say yes, some say no, mainly because the redox potential is not sufficient. This would ignore the presence of thiol reductase enzymes. The single chain antibody fusion protein-toxins are quite toxic. The linkage there is clearly through peptide bonds (they are fusion proteins) so the easiest response to this question is that there is proteolytic degradation. Since saporin is tremendously resistant to proteases, it can't be stopped.

Targeting Teaser Solution

The solution to the puzzle was:

Jumbles: CHOLINE
SCHIZOPHRENIA
FUNCTION
INTERNEURONS
INFUSION

How the intern thought a future of science would impact her future.

Answer: SHE WOULD LIVE A... LIFE OF PI.

Solve this quarter's teaser at www.ATSbio.com/news/13q2_teaser.html.

Congratulations to the puzzle solvers from last quarter. Each winner has received $100 credit towards research product purchases from Advanced Targeting Systems.

WINNERS: Ruth Stornetta, Univ Virgina, Charlottesville, VA * Glenn H. Kageyama, Cal Poly Pomona Univ, Pomona, CA * Donna Bielinski, USDA Human Nutrition Center at Tufts Univ, Boston, MA * Peter Syapin, Texas Tech Univ HSC, Lubbock, TX * Seto Chice, SUNY-HSC, Brooklyn, NY

Targeted Delivery of Cytotoxic Agents to Tumor Cells

(continued from page 1)

melanoma cancer, but with antibody therapy only producing modest results.

Mouse monoclonal R24 antibody (IgG3), directed against ganglioside GD3, is a validated tumor targeting agent that shows strong cell surface reactivity for a range of human melanoma cell lines and other epithelial cancer tumor cells.[8] We demonstrated that in different cell lines the R24 antibody to GD3 is rapidly endocytosed after binding to the disialo ganglioside at the cell surface, sorted to early endosomes and later accumulated in recycling endosomes (Fig. 1).[9] Consequently, its rapid internalization in cells precludes its use as a "naked therapeutic antibody," because when internalized it cannot link to pathways of complement- or cellular-dependent anticancer activities.

We took advantage of the internalization feature of R24 antibody for selective delivery of saporin to GD3-expressing cells.[10] This was carried out by mixing the R24 antibody with Mab-ZAP (Cat. #IT-04), a goat anti-mouse IgG antibody linked to saporin, The targeted toxin was found to be specifically cytotoxic for GD3-expressing cells [human (SK-Mel-28), mouse (B16) melanoma cells and CHO-K1 cells] grown on 2D monolayers. To estimate the potential antitumor activity of the R24/Mab-ZAP complex, we also evaluated the effect of the targeted toxin on clonogenic growth of cells in attachment-free conditions. Briefly, cells expressing the ganglioside GD3 were grown in semi-solid medium for seven days to allow the formation of colonies containing approximately 60-80 cells. Then, cells were exposed to the R24/Mab-ZAP complex and the size of the colony was scored at different times. Surprisingly, a drastic growth inhibition of SK-Mel-28 melanoma cells was reached after only 3 days of treatment (Fig. 2). In contrast, cell colonies continued to grow with the same concentration of the targeted toxin, but in the absence of the R24 antibody, or in the absence of both targeted toxin and R24, undoubtedly indicating the specificity of the effect observed.

Antibody-drug conjugates are emerging as highly effective therapies for cancer. From our studies, the ganglioside GD3 has emerged as a novel and attractive class of cell surface molecule (glycosphingolipid) for targeted delivery of drugs. This is due to its accessibility, low expression in normal cells, high expression in many tumor cells (mainly of neuroectodermal and epithelial origin) and for its capacity to undergo endocytosis after binding with extracellular ligands such as antibodies. We believe that it is possible to potentiate the cytotoxic properties of R24 on target cells by linking it to other drugs such as paclitaxel, which will aid in bypassing multiple drug resistance mediated by the p-glycoprotein pumps.

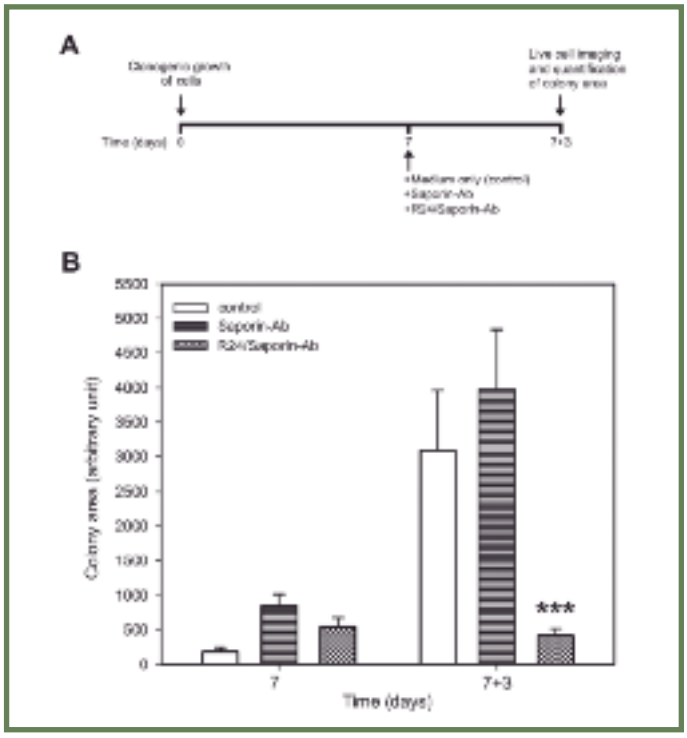


Figure 2. Selective delivery of saporin via R24 antibody drastically reduces the clonogenic growth of human SK-Mel-28 melanoma cells. A) A schematical representation of the experimental procedure used in B. B) SK-Mel-28 cells (50-80 cells) were grown in 24-well plates previously coated with 0.5% agar in culture medium. Cells were maintained at 37ºC until cell colonies appeared (7 days). Then, cells were exposed for 3 days (7+3 days) to 0.95 nM Mab-ZAP (Saporin-Ab) or 30 nM R24/0.95 nM Mab-ZAP (R24/Saporin-Ab). Quantification was performed at 7 and 7+3 days. SK-Mel-28 cells maintained only with medium were used as negative control (control). Results were analyzed by ANOVA followed by Tukey's multiple comparison test. Results are given as means ±S.E. Note that the clonogenic growth of SK-Mel-28 cells was severely affected only in the presence of the R24/Saporin-antibody complex (***$p<0.0001$ with respect to the control condition at 7+3 days). Modified from Ref. 10.

References

1. Daniotti JL *et al.* (2011) *IUBMB Life* 63:513-520.
2. Crespo PM *et al.* (2004) *J Biol Chem* 279:47610-47618.
3. Maccioni HJ *et al.* (1999) *Biochim Biophys Acta* 1437:101-118.
4. Crespo PM *et al.* (2010) *J Biol Chem* 285:29179-29190.
5. Sonnino S *et al.* (2010) *FEBS Lett* 584: 1914-1922.
6. Vilcaes AA *et al.* (2011) *J Biol Chem* 286:31437-31446.
7. Lo AS *et al.* (2010) *Clin Cancer Res* 16:2769-2780.
8. Pukel CS *et al.* (1982) *J Exp Med* 155:1133-1147.
9. Iglesias-Bartolome R *et al.* (2006) *FEBS J* 273:1744-1758.
10. Torres Demichelis V *et al.* (2013) *PLoS One* 8:e55304.

Targeting Tools: Featured Products

FabFc-ZAP: A New Secondary Conjugate

Background of General Preparation and Components:

Over the last few years, riding on the coattails of the established customer base for the ZAP line of secondary conjugates, Fab-ZAP has become the most popular ATS product group in the catalog. Combining species-specific monovalent secondary antibodies with the cytotoxic ribosome-inactivating protein, Saporin, the Fab-ZAP line of products provides researchers with an ideal tool for quick diagnostic analysis of primary antibody internalization with their predicted receptors without having to worry about theoretical false-positive non-specific cell death via bivalent antibody capping.

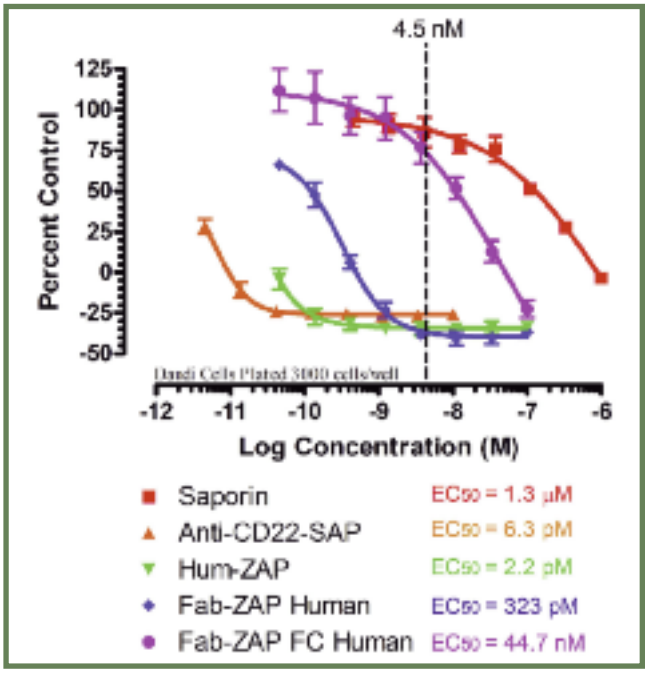
One drawback that ATS has recently addressed within the Fab-ZAP product line is the use of these secondary conjugates with B-cells, particularly popular are those of non-Hodgkin's lymphoma. B-cells express endogenous surface immunoglobulins (sIg), mostly in the form of monomeric IgM that play a significant role in antigen recognition and immune-response activation. The sIg is anchored to the B-cell surface in both naïve and activated B-cells by the Fc region of the molecule while the Fab portion of the sIG could be bound by external human secondary antibodies, including those used in the ATS secondary ZAP conjugates, resulting in unintended B-cell death.

ATS has now released for sale Fc specific Fab-ZAP conjugates for use with two species of primary antibodies, human and mouse. The secondary antibody used in the Fc-specific Fab-ZAP will react only with the Fc portion of the IgG heavy chain. These conjugates have been tested by ELISA and/or absorbed against Fab fragments. These new **FabFc-ZAP products can be used in B-cell research** with the confidence that cell death is primary antibody-mediated.

Methods of Cytotoxicity Assessment:

To test the new material, we first focused on the human specific versions and performed a 5-day *in-vitro* cytotoxicity assay to compare our already established line of secondary conjugates Hum-ZAP (Cat. #IT-22), and Fab-ZAP Human (Cat. #IT-51) vs. the newly developed FabFc-ZAP Human (Cat. #IT-65), on Daudi cells, a human B-lymphoblast. The assay would demonstrate that the specificity of this new product line can remedy issues seen with Hum-ZAP and Fab-ZAP recognizing endogenous surface immunoglobulins and unintentionally eliminating cells to provide a new tool in B-lymphocyte research.

I live life. . .
Gangsta-style.



FabFc-ZAP, human
available individually (IT-65)
available as a kit (KIT-65) including Goat IgG-SAP (IT-19)

(For the complete protocol visit our website: www.atsbio.com/protocols.)

Results and Discussion:

Analysis of these data demonstrate a firm correlation with what was expected: Hum-ZAP and Fab-ZAP Human are excellent at binding human IgG, so much so that we don't recommend their use with B-cells because they exhibit unintended binding with endogenous surface immunoglobulins.

The conjugate Hum-ZAP displayed the most B-cell cross-reactivity with an EC_{50} of 2.2 pM, followed by Fab-ZAP Human with an EC_{50} of 323 pM. **The EC_{50} for the new Fc-specific FabFc-ZAP was calculated at 44.7 nM, showing to be less potent by a magnitude of 100-fold under the previous Fab-ZAP Human and a 20,000-fold difference under Hum-ZAP.**

The recommended use of our secondary conjugates is approximately 4.5 nM. This concentration point has been depicted on the graph by a dotted-line and clearly demonstrates the difference between the newly developed FabFc-ZAP (Purple Line) vs. previous secondary conjugates and controls and their reaction when specifically studying B-lymphoblasts.

Our goal is to continue providing our customers with the tools they need to advance their science and we look forward to the future challenges. It's these challenges that give us the chance to work with our customers to improve our products and create new ones.

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

10451 Roselle Street #300
San Diego, CA 92121

PRESORTED STD.
U.S. POSTAGE
PAID
SAN DIEGO, CA
PERMIST # 2686

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.

IDECREAM
◯☐☐☐☐☐☐☐

MULUTRICE
◯☐☐☐◯☐☐☐☐

FARCEUS
◯☐☐☐☐☐◯

HEPARTY
◯☐◯☐☐☐☐

ANOMALEM
☐☐☐☐◯◯☐◯

What the graduate student learned when she took a course on "Creativity in the Laboratory".

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

ANSWER:
SHE LEARNED THE. . . ◯◯◯ ◯◯ ◯◯◯◯◯◯◯◯.

WIN $100.00

Limit one entry per laboratory. Credits expire after one year.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by June 15, 2013.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

Please correct the address information above and provide the following:

Your Name:
Phone:
Fax:
Email:

Jul-Aug-Sep 2013
Volume 14, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- Optogenetics, Brain Activity Mapping Project; Spring Brain Conference 25th Anniversary *(page 2)*
- Targeting Talk: Streptavidin-ZAP New Calculators *(page 5)*
- Recombinant Isolectin B4 (patent pending) *(page 7)*
- Targeting Teaser Win a Tote Bag! *(page 8)*

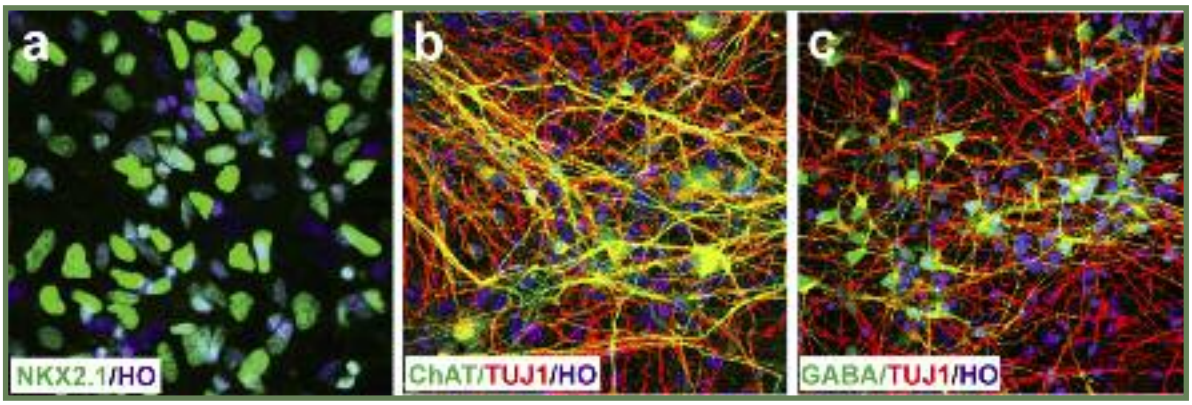
Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Medial ganglionic eminence-like cells derived from human embryonic stem cells correct learning and memory deficits in an NGFr-lesioned mouse model

Liu Y, Weick JP, Liu H, Krencik R, Zhang X, Ma L, Zhou GM, Ayala M, Zhang SC.
Waisman Center, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin, USA. and Human Anatomy and Histology, Fudan University Shanghai Medical School, Shanghai, China.

Basal forebrain neurons including cholinergic neurons and GABA interneurons originate from the medial ganglionic eminence (MGE),[1] and play key roles in learning and memory. Dysfunction of MGE progenies results in learning and memory deficits, and may associate with many diseases including Alzheimer's disease, Down syndrome, and dementia, none of which has an effective cure at present.[2-5] Directed differentiation of basal forebrain neurons from human pluripotent stem cells (hPSC), including embryonic stem cells (hESC)[6] and induced pluripotent stem cells (hiPSC),[7] might become a potential treatment for these learning and memory deficit diseases.



hPSCs can be maintained and proliferated long-term in a cell culture system. Under certain conditions, hPSCs can be differentiated into many types of cells of the human body, including neurons.[8-10] The process of differentiation to a certain type of neuron mimics neural development *in vitro*.[8] Previously, we generated various neurons from hPSCs, including cortical glutamatergic neurons,[11] midbrain dopaminergic neurons,[12,13] spinal cord motor neurons,[11,14] and striatal GABA neurons[15] by following each developmental principle.

MGE cells located in the ventral forebrain express transcriptional factors FOXG1 and NKX2.1.[16] Sonic hedgehog (SHH) is secreted from notochord and forms a concentration gradient from ventral to dorsal.[17,18] Therefore, a high concentration of SHH is required for generation of MGE cells. We applied 1000 ng/ml of SHH at an early timepoint to pattern hPSCs to nearly pure MGE progenitors. These progenitors can be further differentiated into cells that are electrophysiologically functional with 40% cholinergic neurons and 50% GABA interneurons *in vitro*.[19]

(continued on page 6)

Fig 1. a) After treatment with a high concentration of Sonic hedgehog, most cells (Hoechst-HO, **blue**) become NKX2.1-positive (**green**), illustrating MGE identity.
b) *In vitro*, MGE cells differentiate to cholinergic neurons (ChAT, **green**) that coexpress neural marker TUJ1 (**red**).
c) *In vitro*, MGE cells differentiate to GABA interneurons (GABA, **green**) that coexpress neural marker TUJ1 (**red**).

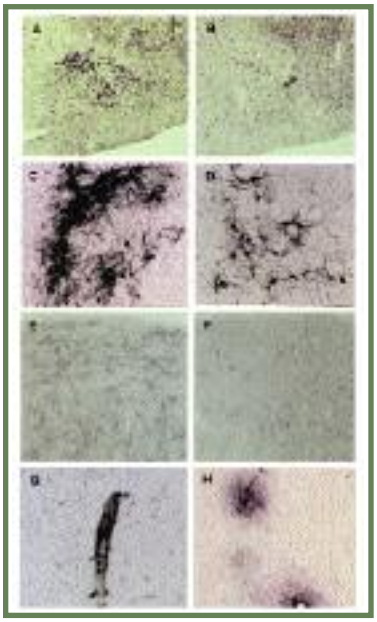
Targeted Toxins, Optogenetics, and the BRAIN Initiative

Contributed by Brian J. Russell

The newly invigorated BRAIN initiative (Brain Research through Advancing Innovative Neurotechnologies, also known as the Brain Activity Map Project) has placed a premium on understanding the role of each neuron within the human brain. This effort has been our focus for some time via our Targeted Toxin technology. From the mid-nineties papers using 192 IgG-SAP (Cat. #IT-01) to the subsequent work with the multiple-species version ME20.4-SAP (Cat. #IT-15), Advanced Targeting Systems (ATS) has been instrumental in helping experimental neuroscientists discover the function of cholinergic neurons of the basal forebrain and projecting to the hippocampus and cortex. Neither the BRAIN initiative nor ATS is interested in limiting the studies to cholinergic neurons and ATS has been successful in providing a variety of Targeted Toxins for use throughout the brain and nervous systems.

As our customers already know, Targeted Toxins are very cost-effective for studying neuronal pathways. The technology is rapid, conclusive, and available for use in most common lab species. In many scenarios the permanent lesion of a Targeted Toxin can provide a better model than transient lesion techniques. Developing technologies often suffer from difficult-to-interpret negative data and will benefit from the established and growing history of published literature on toxin conjugates, which can be used as positive controls.[1]

Optogenetics is a technology that is making amazing progress in helping to understand the intricacies of neuronal pathways. Despite the difficulties and time associated with viral vector construction and opsin introduction to the neurons of interest, the ability to use different opsins to either excite or inhibit neurons in a temporary manner is a very useful technique. However, the equipment and techniques necessary to effectively use optogenetics may prove to be an obstacle in some situations. What will be interesting to see is how the technology of Optogenetics attempts to clear the hurdle presented by larger species such as primates and the ultimate goal of the BRAIN initiative, which is to map the human brain. Particular difficulties include stimulating neurons that are deep inside the larger brains of primates and any potential long-term damage associated with repeated probing with optical fibers.[2] Lastly, as the pharmaceutical industry embraces toxin conjugates for new treatment options, the clinical uses for optogenetics are less clear due to ethical and complexity considerations.



Histologic demonstration of the nbm lesion and resultant Aβ deposition. Rabbit nbm area in control (A) and in treated animals (B), stained for AChE. Note the marked depletion of nbm neurons in the latter. Rabbit nbm in control (C) and treated (D) animals, stained IHC for p75 NTR. Note depletion of neurons in the treated animal. Frontal cortex from control (E) and treated (F) animals, stained for AChE. Note eradication of cholinergic fibers in the latter. G,H: Aβ deposition in cerebral cortex of lesioned animals. Aβ is deposited in blood vessel walls (G) and in the perivascular neuropil (H).

References:

1. Baxter MG, Bucci DJ. (2013) Selective immunotoxic lesions of basal forebrain cholinergic neurons: twenty tears of research and new directions. *Behav Neurosci*, in press.
2. Gerits A, Vanduffel W. (2013) Optogenetics in primates: a shining future? *Trends Genet*, in press.

Spring Brain Conference: 25th Anniversary

The Spring Brain Conference will have its 25th Anniversary in beautiful Sedona Arizona March 19-22, 2014. The confirmed speakers offer a fascinating look at what's happening in the Neurosciences. As a keynote speaker, **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. **Mark A. Hoon** will lead a session on somatosensory issues, including his recent publication in *Science* on itch and how it works (see summary on Page 3). **Hans Keirstead**, who began the first stem cell clinical trial, will lead a session on, well, great stuff that is -- or will be -- in the clinic for problems in the brain or spinal cord.

If you have a fascinating story that needs telling, send Doug Lappi a message on the Spring Brain Conference website. In any case, plan on coming to Sedona for fun and great science. Last year's Program, along with visitor information and pretty pictures, can be viewed at **www.springbrain.org**.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Medial ganglionic eminence-like cells derived from human embryonic stem cells correct learning and memory deficits.

Liu Y, Weick JP, Liu H, Krencik R, Zhang X, Ma L, Zhou GM, Ayala M, Zhang SC.
Nat Biotechnol 31(5):440-447, 2013.

Progenitor cells were transplanted into mice that had received 1.5 µg of mu p75-SAP (Cat. #IT-16) into the medial septum. *See cover article*.

The cells and circuitry for itch responses in mice.

Mishra SK, Hoon MA.
Science 340(6135):968-971, 2013.

Although previous work implicated neurons expressing the GRP (gastrin-releasing peptide) receptor were in the pruritic, or itch pathway, transgenic mice lacking natriuretic polypeptide b (Nppb) were almost completely insensitive to itch. Using the custom conjugate Nppb-SAP, the authors eliminated itch in response to a wide range of pruritic substances in normal mice through the administration of 5 µg of conjugate into the intrathecal space. Even after this lesion, the scratching response to intrathecal GRP was not changed, indicating that the role of GRP is at a later stage than previously hypothesized.

A Novel Model for Evaluating Therapies Targeting Human Tumor Vasculature and Human Cancer Stem-like Cells.

Burgos-Ojeda D, McLean K, Bai S, Pulaski H, Gong Y, Silva I, Skorecki K, Tzukerman M, Buckanovich RJ.
Cancer Res 73(12):3555-3565, 2013.

Targeting tumor vascular markers (TVM) is difficult since the vasculature expression profile of tumor types tends to be very different. The authors established a human embryonic stem cell-derived teratoma and tested it as a model for TVM expression by challenging primary human MSCs *in vitro* and in an *in vivo* mouse tumor model created with anti-human Thy1 plus Streptavidin-ZAP (Cat. #IT-27).

Striatal patch compartment lesions alter methamphetamine-induced behavior and immediate early gene expression in the striatum, substantia nigra and frontal cortex.

Murray RC, Gilbert YE, Logan AS, Hebbard JC, Horner KA.
Brain Struct Funct Epub2013.

In this work, the authors investigated the function of the patch compartment in abnormally repetitive motor actions (stereotypy) in response to a psychostimulant such as methamphetamine (meth). Rats received bilateral injections of Dermorphin-SAP (Cat. #IT-12; 17 ng) into the rostral striatum. When treated with meth, lesioned animals displayed reduced stereotypy, increased motor activity, and enhanced c-Fos expression.

Photochemical internalization (PCI) of immunotoxins targeting CD133 is specific and highly potent at femtomolar levels in cells with cancer stem cell properties.

Bostad M, Berg K, Hogset A, Skarpen E, Stenmark H, Selbo PK.
J Control Release 168(3):317-326, 2013.

Targeted therapies for cancer can be trapped in the lysosome and compartmentalized away from the target. Photochemical internalization is a method to increase the efficacy of these compounds by releasing the therapeutic portion of the molecule from the endocytic vesicles to the cytosol by the use of light. The authors demonstrate this method on cells expressing the cancer stem cell marker CD133. Biotinylated antibodies against CD133 were combined with Streptavidin-ZAP (Cat. #IT-27) and applied to cell lines.

Cortical cholinergic input is required for normal auditory perception and experience-dependent plasticity in adult ferrets.

Leach ND, Nodal FR, Cordery PM, King AJ, Bajo VM.
J Neurosci 33(15):6659-6671, 2013.

In order to study how cholinergic input from the nucleus basalis affects auditory perception and learning, the authors injected a total of 35.2 ng of ME20.4-SAP (Cat. #IT-15) into the nucleus basalis in each hemisphere of ferrets. Based on several learning tasks, the data suggest that these cholinergic inputs aid in the perception of sound source location and aid in the adaptation of the auditory system to changes in spatial cues.

Cutting Edge: memory regulatory t cells require IL-7 and not IL-2 for their maintenance in peripheral tissues.

Gratz IK, Truong HA, Yang SH, Maurano MM, Lee K, Abbas AK, Rosenblum MD.
J Immunol 190(9):4483-4487, 2013.

Recently a new class of regulatory T cells (mTregs) were found that persist in non-lymphoid tissues and are involved in suppressing autoimmune responses. In order to examine the roles that IL-2 and IL-7 play in the development and regulation of mTregs, the authors used genetic deletion, adoptive T-cell transfer, and *in vivo* neutralization techniques. 5 µg of intravenous OX7-SAP (Cat. #IT-02) per mouse was used to deplete CD90.1-positive cells during the adoptive transfer experiment. It was found that IL-7 is essential for the steady-state maintenance of mTregs in skin.

Combining phenotypic and proteomic approaches to identify membrane targets in a 'triple negative' breast cancer cell type.

Rust S, Guillard S, Sachsenmeier K, Hay C, Davidson M, Karlsson A, Karlsson R, Brand E, Lowne D, Elvin J, Flynn M, Kurosawa G, Hollingsworth R, Jermutus L, Minter R.
Mol Cancer 12:11, 2013.

The authors investigated a phenotypic antibody screening technique, in which antibodies are selected by function rather than target specificity. One facet of the screening procedure for hybridomas generated using a cancer cell line as antigen was the use of Mab-ZAP (Cat. #IT-04) to assess cell binding and internalization.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Immunolesion-induced loss of cholinergic projection neurons promotes beta-amyloidosis and tau hyperphosphorylation in the hippocampus of triple-transgenic mice.

Hartig W, Saul A, Kacza J, Grosche J, Goldhammer S, Michalski D, Wirths O.

Neuropathol Appl Neurobiol Epub2013.

3xTg transgenic mice were treated with 2 µg of mu p75-SAP (Cat. #IT-16) into the right lateral ventricle to eliminate cholinergic neurons in the basal forebrain. These mice already have age-dependent β-amyloidosis and tau hyperphosphorylation. This new model supplies a potential framework in which to study the entire pathology of Alzheimer's disease.

Cholinergic basal forebrain structures are involved in the mediation of the arousal effect of noradrenaline.

Lelkes Z, Porkka-Heiskanen T, Stenberg D.

J Sleep Res Epub2013.

Wakefulness is enhanced by the injection of noradrenaline into the basal forebrain, but it has not been clear whether cholinergic or non-cholinergic neurons are involved. 230 ng of 192-IgG-SAP (Cat. #IT-01) was administered to the horizontal diagonal band/substantia innominata/ magnocellular preoptic nucleus of rats. Upon treatment with methoxamine, lesioned animals lost the non-REM sleep-suppressing effect, but the REM sleep-suppressing effect remained intact.

An environment-dependent modulation of cortical neural response by forebrain cholinergic neurons in awake rat.

Ariffin MZ, Chang LS, Koh HC, Low CM, Khanna S.

Brain Res 1513:72-84, 2013.

Rats received 168 ng of 192-IgG-SAP (Cat. #IT-01) into the medial septum and induction of c-Fos expression in response to either familiar or novel stimuli was measured.

Plasmin induces intercellular adhesion molecule 1 expression in human endothelial cells via nuclear factor-kappaB/mitogen-activated protein kinases-dependent pathways.

Li Q, Syrovets T, Simmet T, Ding J, Xu J, Chen W, Zhu D, Gao P.

Exp Biol Med (Maywood) 238(2):176-186, 2013.

Intracellular adhesion molecule 1 (ICAM-1) mediates inflammatory cell migration – an early step in atherosclerosis. The authors investigated an inflammatory cascade activated by plasmin using a variety of methods, including flow cytometry with anti-mouse IgG-FITC (Cat. #FL-07) and anti-rabbit IgG-FITC (Cat. #FL-04).

Evaluating the Role of Neuronal Nitric Oxide Synthase-Containing Striatal Interneurons in Methamphetamine-Induced Dopamine Neurotoxicity.

Fricks-Gleason AN, Keefe KA.

Neurotox Res Epub2013.

Using the fact that neuronal nitric oxide synthase (nNOS)-containing neurons in the striatum express the substance P receptor, the authors injected four locations in the striatum with 3 ng each of SSP-SAP (Cat. #IT-11). Blank-SAP (Cat. #IT-21) was used as a control. Although there was a significant loss of nNOS-containing neurons, the lesions did not attenuate NO production.

The role of visual cortex acetylcholine in learning to discriminate temporally modulated visual stimuli.

Minces VH, Alexander AS, Datlow M, Alfonso SI, Chiba AA.

Front Behav Neurosci 7:16, 2013.

In order to examine some of the minor differences in the temporal structure of stimuli, the authors bilaterally injected 37.5 ng of 192-IgG-SAP (Cat. #IT-01) between the lambda and bregma of rats. This injection reduced acetylcholine projections to the visual cortex. Loss of that cholinergic input impaired the ability of the lesioned animals to perform fine discriminations, but previously learned discriminations remained unimpaired.

Photochemical internalization of CD133-targeting immunotoxins efficiently depletes sarcoma cells with stem-like properties and reduces tumorigenicity.

Stratford EW, Bostad M, Castro R, Skarpen E, Berg K, Hogset A, Myklebost O, Selbo PK.

Biochim Biophys Acta 1830(8):4235-4243, 2013.

In this work the authors used photochemical internalization (PCI) to facilitate local cytosolic toxin release from various biotinylated-anti-CD133 antibodies coupled with Streptavidin-ZAP (Cat. #IT-27) in SW872 cells. This technique demonstrates potential for non-invasive sarcoma therapy.

Combinatorial Treatment of Tart Cherry Extract and Essential Fatty Acids Reduces Cognitive Impairments and Inflammation in the mu-p75 Saporin-Induced Mouse Model of Alzheimer's Disease.

Matchynski JJ, Lowrance SA, Pappas C, Rossignol J, Puckett N, Sandstrom M, Dunbar GL.

J Med Food 16(4):288-295, 2013.

The authors investigated the efficacy of a combinatorial therapy, Cerise Total-Body Rhythm (TBR) by treating mice with TBR prior to and following icv administration of 0.8 µg of mu p75-SAP (Cat. #IT-16).

(continued on page 5)

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

Targeting Talk: Product Questions

Q: Some suppliers sell their streptavidin conjugates in amounts given as streptavidin equivalents. Is that also the case for your product, Streptavidin-ZAP? What is important to me is to know what the molar concentration of streptavidin conjugate is, and the volume of your preparation.

A: The molar concentration of Streptavidin-ZAP (Cat. #IT-27) will depend on the lot; the accompanying data sheet will contain the molecular weight. We recommend that you mix Streptavidin-ZAP and your biotinylated material at equimolar concentrations. For our in-house *in vitro* quality control assays, we make a stock vial containing both 1 μM of biotinylated material and 1 μM of Streptavidin-ZAP diluted in media in 150 μl total volume. From this stock vial, we add 10 μl to each well of a plate containing cells in 90 μl volumes, which then dilutes the stock material to its correct concentration of 100 nM. Check out the calculators on our website: **www.atsbio.com/calculations.html**

Calculate Volume Required to Dilute a Solution
Calculate Molarity of a Solution
Calculate Volume of a Solution
Calculate Mass of a Solution
Convert Between Moles and Grams
Convert Molar Units
Convert Liter Units

Q&A Products

Streptavidin-ZAP (IT-27)
Custom Saporin Conjugates

Q: So, each mole of streptavidin will bind 4 moles of biotin?

A: Streptavidin-ZAP was created for use as an initial diagnostic step with biotinylated targeting agents, before moving on to a direct linkage between the optimal targeting agent and Saporin. The biotin-streptavidin interaction should be considered a linker; the major players are the targeting agent and Saporin. The targeting agent to Saporin ratio is kept at 1:2 M. When pre-mixing the biotinylated moiety with Streptavidin-ZAP in an equimolar ratio the ability of Streptavidin-ZAP to bind up to 4 biotins ensures that most of the biotinylated moiety will have Streptavidin-ZAP attached (no free biotinylated moiety). Streptavidin equivalents would not be appropriate as the Saporin moiety in Streptavidin-ZAP is the primary focus of the technology.

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

Targeting Topics: Recent Scientific References

(continued from page 5)

BB2 bombesin receptor-expressing spinal neurons transmit herpes-associated itch by BB2 receptor-independent signaling.

Sasaki A, Adhikari S, Andoh T, Kuraishi Y.
Neuroreport Epub2013.

Using a skin rash model created by inoculating mice with human herpes virus, bombesin receptor-expressing spinal neurons were lesioned intrathecally with 400 ng of Bombesin-SAP (Cat. #IT-40). Lesioned animals displayed reduced scratching, but licking (due to pain) was not reduced.

Time Course Study of Targeted Ablation of KNDy Neurons, but not Tyrosine-Hydroxylase Neurons, in the Rat Arcuate Nucleus Using a Neurokinin B-Saporin.

Helena CV, Kalil B, Anselmo-Franci JA, Bertram R.
Endocr Rev 34 (03_MeetingAbstracts):OR47-5, 2013.

Ovariectomized rats were given bilateral injections of NK3-SAP (Cat. #IT-63) into the arcuate nucleus for a time course study of KNDy neuron loss. Blank-SAP (Cat. #IT-21) was used as a control.

State-dependent contribution of the hyperpolarization-activated na+/k+ and persistent na+ currents to respiratory rhythmogenesis in vivo.

Montandon G, Horner RL.
J Neurosci 33(20):8716-8728, 2013.

The hyperpolarization-activated cation current in the preBötzinger complex (preBötC) was identified as a critical component of respiratory rhythm. An anti-neurokinin 1 receptor antibody (Cat. #AB-N04 replaced by Cat. #AB-N33AP) at a 1:1000 dilution was used to identify preBötC neurons in the brainstem.

Correcting learning and memory deficits in an NGFr-lesioned mouse model

(continued from page 1)

In order to further determine whether the hPSC-derived MGE progenitors are functional *in vivo*, we generated a mouse model with learning and memory deficits by using mu p75-SAP (Cat. #IT-16). This targeted toxin consists of an affinity-purified rabbit polyclonal antibody specific to the mouse low affinity nerve growth factor receptor (NGFr) conjugated to the ribosome-inactivating protein, saporin. We injected 1.5 µg of mu p75-SAP into the medial septum; another group of mice were injected with artificial cerebral spinal fluid (aCSF) as sham lesion control. Two weeks after injection we checked the expression levels in the medial septum of cholinergic neurons and GABA interneurons: almost all the cholinergic neurons were killed by mu p75-SAP; and parvalbumin (PV, one of GABA interneuron subtypes) neurons were also decreased.* When tested in the Morris water maze, the learning and memory ability of lesioned mice was significantly decreased as compared to sham mice.

After one month post-lesion, we injected MGE progenitors into two sides of the hippocampus that are in the area of the medial septal neurons. VSP (ventral spinal progenitors) were injected as a cellular transplantation control, and aCSF as a surgery control. Human MGE progenitors differentiated into cholinergic neurons and GABA interneurons in the mouse hippocampus and formed functional connections with host neurons as tested by slice electrophysiology and immunostaining. Six months after transplantation, using the Morris water maze test, the group receiving MGE progenitors had significantly increased learning and memory ability, while control groups did not learn well. We conclude that MGE cells have a function *in vivo* to ameliorate learning and memory deficits in lesioned mice.

**Editor's Note:* *In order to establish this model properly, a higher dose than usual of mu p75-SAP was used which caused some non-specific damage. For standard lesioning applications, ATS recommends that a control conjugate (non-targeted agent conjugated to saporin) be used in experiments to guarantee there is no non-specific cell death.*

References

1. Sussel L, Marin O, Kimura S, Rubenstein JL. (1999) Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development* 126:3359-3370.
2. Oliveira AA, Jr., Hodges HM. (2005) Alzheimer's disease and neural transplantation as prospective cell therapy. *Curr Alzheimer Res* 2:79-95.
3. Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR. (1982) Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* 215:1237-1239.
4. Pang KC, Jiao X, Sinha S, Beck KD, Servatius RJ. (2010) Damage of GABAergic neurons in the medial septum impairs spatial working memory and extinction of active avoidance: Effects on proactive interference. *Hippocampus* 21:835-846.
5. Dwyer TA, Servatius RJ, Pang KC. (2007) Noncholinergic lesions of the medial septum impair sequential learning of different spatial locations. *J Neurosci* 27:299-303.
6. Thomson JA, Itskovitz-Eldor J, Shapiro SS, *et al*. (1998) Embryonic stem cell lines derived from human blastocysts. *Science* 282:1145-1147.
7. Takahashi K, Tanabe K, Ohnuki M, *et al.* (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131:861-872.
8. Zhang SC. (2006) Neural subtype specification from embryonic stem cells. *Brain Pathol* 16:132-142.
9. Yamanaka S. (2009) A fresh look at iPS cells. *Cell* 137:13-17.
10. Liu Y, Zhang SC. (2010) Human stem cells as a model of motoneuron development and diseases. *Ann N Y Acad Sci* 1198:192-200.
11. Li XJ, Hu BY, Jones SA, *et al.* (2008) Directed differentiation of ventral spinal progenitors and motor neurons from human embryonic stem cells by small molecules. *Stem Cells* 26:886-893.
12. Xi J, Liu Y, Liu H, Chen H, Emborg ME, Zhang SC. (2012) Specification of midbrain dopamine neurons from primate pluripotent stem cells. *Stem Cells* 30:1655-1663.
13. Yan Y, Yang D, Zarnowska ED, *et al.* (2005) Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. *Stem Cells* 23:781-790.
14. Li XJ, Du ZW, Zarnowska ED, *et al.* (2005) Specification of motoneurons from human embryonic stem cells. *Nat Biotechnol* 23:215-221.
15. Ma L, Hu B, Liu Y, *et al*. (2012) Human embryonic stem cell-derived GABA neurons correct locomotion deficits in quinolinic acid-lesioned mice. *Cell Stem Cell* 10:455-464.
16. Flames N, Pla R, Gelman DM, Rubenstein JL, Puelles L, Marin O. (2007) Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. *J Neurosci* 27:9682-9695.
17. Rubenstein JL, Shimamura K, Martinez S, Puelles L. (1998) Regionalization of the prosencephalic neural plate. *Ann Rev Neurosci* 21:445-477.
18. Wilson SW, Rubenstein JL. (2000) Induction and dorsoventral patterning of the telencephalon. *Neuron* 28:641-651.
19. Liu Y, Weick JP, Liu H, *et al.* (2013) Medial ganglionic eminence-like cells derived from human embryonic stem cells correct learning and memory deficits. *Nat Biotechnol* 31:440-447.

Targeting Tools: Featured Products

*Recombinant Isolectin B4 (IB4)**

Contributed by Matthew Kohls

Isolectin B4 (IB4) is a tetrameric protein isolated from *Griffonia simplicifolia* seeds. Although one of its first uses was the agglutination of B-type red blood cells,[1] it has also been used extensively in the neurosciences because the alpha-galactosyl residues bound by IB4 are present on a variety of cell populations such as the non-peptidergic afferents from the dorsal root ganglia to the spinal cord and subpopulations of somatosensory neurons.[2] Purification of the native protein is a relatively straightforward process, but *Griffonia* contains 5 similar isolectins, and producing a completely homogeneous lot of IB4 with no contamination of the other lectins is challenging.

rIB4
available unconjugated (PR-02);
available as part of a Targeted Toxin, rIB4-SAP (IT-10);
and in a kit (KIT-10) including rIB4-SAP (IT-10) and
rIB4 mixed with Unconjugated Saporin

The main supply of *Griffonia* seeds is from Africa, mainly Ghana, Liberia, Togo, and the Ivory Coast. These seeds are also a source of 5-hydroxytryptophan (5-HTP) which is in high demand in the dietary supplement market as an antidepressant, an appetite suppressant, and a sleep aid. As the demand for 5-HTP has gone up, the growers have begun treating the seeds such that they will not germinate, protecting the source of the seeds and the income of the growers. Although 5-HTP can still be purified from treated seeds, IB4 yield is drastically reduced, and the activity is significantly lower than previous lots.

Advanced Targeting Systems has circumvented these issues by cloning IB4 from *Griffonia* genomic DNA.* The cloned protein is now expressed in *E. coli* at high levels, and can be purified in a single step – the final product is a homogeneous protein with equivalent activity to native IB4 (nIB4) purified from non-treated seeds (Fig 1). Recombinant IB4 (Cat. #PR-02) has been tested in agglutination assays and as a targeted conjugate in cytotoxicity assays. Work is in progress on variations of IB4 such as a GFP fusion protein for use in staining applications.*

The development of a recombinant version of IB4 will provide a steady supply of highly pure protein that is consistent from batch to batch. This new version of IB4 has been shown to have equivalent performance to the native version and is now available for use (Fig 2).

* patent pending

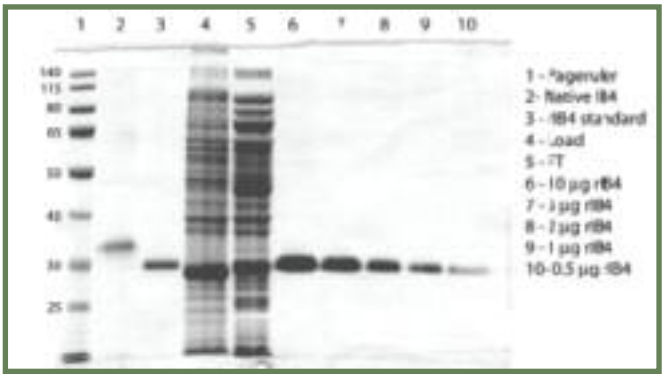


Fig 1. Coomassie stained NuPage gel of large-scale production of rIB4. After a single-step purification of rIB4 over a p-aminobenzyl 1-thio-β-galactopyranoside agarose column, the resulting product is extremely pure, as seen in Lanes 6-10. It can also be seen that the rIB4 in lanes 3 and 6-10 is smaller than the native IB4 in Lane 2, which is glycosylated.

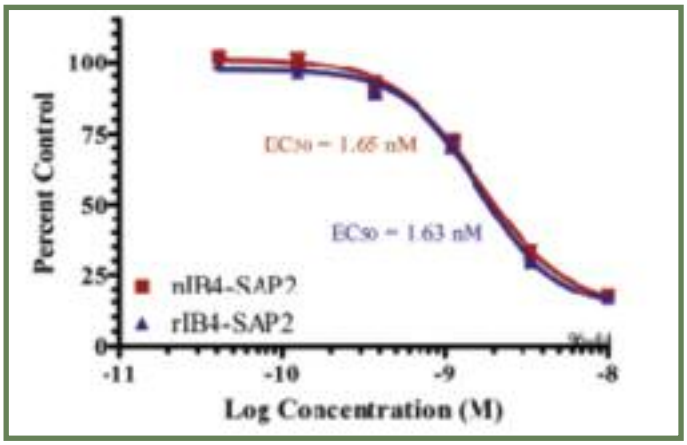


Fig 2. nIB4-SAP and rIB4-SAP conjugates were applied to KNRK cells in a 96-well plate in three separate assays. Various concentrations of the conjugates were incubated with the cells for 72 hours. The conjugates display highly specific cell-killing activity. The conjugate made with the recombinant form of IB4 has the same activity as the conjugate made with the native IB4. The average EC_{50} of IB4-SAP over the three assays was 1.575 nM. The average EC_{50} of rIB4-SAP was 1.619 nM.

References:

1. Hayes CE, Goldstein IJ. (1974) An alpha-D-galactosyl-binding lectin from *Bandeiraea simplicifolia* seeds. Isolation by affinity chromatography and characterization. *J Biol Chem* 249(6):1904-1914.
2. Barabas ME, Kossyreva EA, Stucky CL. (2012) TRPA1 is functionally expressed primarily by IB4-binding, non-peptidergic mouse and rat sensory neurons. *PLoS One* 7(10):e47988.

Gangsta enjoys a rest in his favorite garden spot.

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

10451 Roselle Street #300
San Diego, CA 92121

PRESORTED STD.
U.S. POSTAGE
PAID
SAN DIEGO, CA
PERMIT # 2686

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.

PURPLEOTINT

DEHOGHEG

ROSLAD

CLINGONIAG

BRINYCOME

What the ATS Knockout Mouse did to support himself while earning his PhD in Neuroscience.

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

ANSWER: **HE WORKED AS A . . .** ◯◯◯◯◯ **!**

WIN!

SOLVE the puzzle online with the correct solution by September 15, 2013.

WIN a large, reusable flat-bottom tote bag featuring the ATS Knockout Mouse!

www.atsbio.com/news/13q3_teaser.html

Oct-Nov-Dec 2013
Volume 14, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Role of spinal microglia in the development of morphine-induced hyperalgesia

Contributed by Francesco Ferrini[1] and Yves De Koninck[2,3]

[1]*Department of Veterinary Sciences, University of Turin,10095 Grugliasco, Turin, Italy*
[2]*Institut universitaire en santé mentale de Québec, QC, G1J 2G3, Canada*
[3]*Department of Psychiatry and Neuroscience, Université Laval, Québec, QC, G13 7P4, Canada*

Morphine-induced hyperalgesia and tolerance dramatically limit the use of morphine, especially in chronic diseases. By definition, morphine tolerance is a reduced antinociceptive effect for a given morphine dose, while morphine-induced hyperalgesia is a state of nociceptive sensitization observed in morphine-treated patients.[1,2] It is therefore tempting to postulate that antinociceptive tolerance is set by the decrease in nociceptive threshold due to the hyperalgesia.[3] However, this common view appears to be in contrast with clinical evidence indicating that while increasing the morphine dose can effectively counteract morphine tolerance, the same approach can backfire and worsen pain symptoms in patients with morphine-induced hyperalgesia.[1] In our recent study published in *Nature Neuroscience,*[4] we demonstrated that morphine hyperalgesia and morphine tolerance are mechanistically distinct and that morphine induces hyperalgesia by recapitulating the same maladaptive mechanisms in the spinal cord observed in pathological pain syndromes.

In particular, we addressed the question whether microglia drive morphine-induced hyperalgesia, as the communication between neurons and microglia in the spinal dorsal horn plays a central role in the development of neuropathic pain.[5] The role of microglia in diseases can be tested by using pharmacological tools, such as minocycline, which have been proved to inhibit microglia function.[6] However, the specificity of such approaches to target microglia is debated and direct effects on neuronal activity cannot be ruled out.[7] Therefore, we decided to perform intrathecal injections of a saporin-conjugated antibody against the *(continued on page 6)*

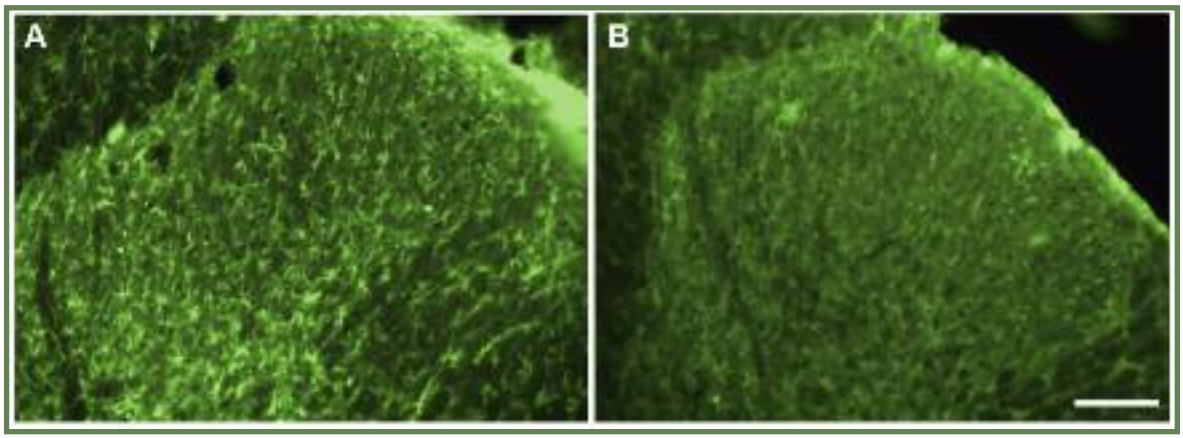


Figure 1. Microglia depletion in the lumbar region of the spinal cord after intrathecal injections of Mac-1-SAP. Microglia are immunohistochemically identified by a mouse CD11b antibody, clone OX-42 (1:500). A) A representative cervical spinal dorsal horn section obtained from a rat which was subcutaneously injected with morphine for 10 days (10 mg/kg) and intrathecally injected with Mac-1-SAP (20 µg) during the last 3 days. B) A representative lumbar spinal dorsal horn section from the same rat showing the decrease in OX-42 staining after Mac-1-SAP treatment. Scale bar 50 µm.

Inside this issue:

Newsletter Highlights

- Spring Brain Conference 25th Anniversary *(page 2)*
- Targeting Talk: Antibody questions. . . answered *(page 5)*
- NEW: ZAP Antibody Internalization Kit *(page 7)*
- Targeting Teaser Win a Tote Bag! *(page 8)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Spring Brain Conference: 25th Anniversary

March 19-22, 2014

The Spring Brain Conference (SBC) will have its 25th Anniversary this March in beautiful Sedona, Arizona. SBC is a broad-brush meeting in which neuroscientists from all disciplines come to meet and exchange ideas in a beautiful environment. The format is designed to allow for extensive discussion with people outside of your immediate field. It is the objective of this conference to bring together scientists with varied backgrounds, interests and approaches to the study of brain function to promote the development of new strategies necessary to better understand the complexities of neural systems. The confirmed speakers for the 2014 meeting offer a fascinating look at what's happening in the Neurosciences.

The meeting starts Wednesday evening with a cocktail reception, introductions of conference speakers and poster presenters and 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*; *Stem Cells in the Clinic* with **Hans Keirstead**, who began the first stem cell clinical trial, as Session Leader; *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**, Session Leader, NIH -- check out Dr. Hoon's recent publication in Science on itch and how it works); *Songbirds and Vocal Learning* with **Stephanie White**, UCLA, as Session Leader, and what these birds tell us about song and speech, synapses, and even disorders such as autism.

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 send a message at the Spring Brain website (www.SpringBrain.org) or stop by the ATS Booth at the Society for Neuroscience meeting in San Diego (November 9-13; Booth 1120).

Come to the Spring Brain Conference 25th Anniversary Social in San Diego

DATE: Sunday, November 10 at the Hilton Gaslamp Hotel (directly across from the San Diego Convention Center)

TIME: 5pm - 7pm

WHAT: Live music, appetizers, beverages: come learn more about SBC and celebrate 25 years of learning!

Email denise@springbrain.org for your free drink ticket and invitation.

Congratulations, Mr. and Mrs. Brian Russell !

ATS is pleased to announce the marriage of Brian Russell to Candilee DeBlase. The happy couple celebrated their nuptials on Saturday, October 5.

Brian is Asst. Research Scientist, Custom Services Guru and Product Manager and has been making great contributions to ATS since October 2000.

Brian and Candi, we wish you both all the best!

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Neurotrophic factors rescue basal forebrain cholinergic neurons and improveperformance on a spatial learning test.

Lee YS, Danandeh A, Baratta J, Lin CY, Yu J, Robertson RT.

Exp Neurol Epub2013.

It is thought that therapeutic treatments of the cholinergic system may be a viable treatment for Alzheimer's disease. In order to examine this hypothesis the authors administered a total of 160 ng of 192-IgG-SAP (Cat. #IT-01) in the form of bilateral injections into the medial septum. The lesioned animals then received 4-week infusions of nerve growth factor, neurotrophin 3, or both into the lateral ventricles. Animals treated with any neurotrophin, either alone or as a combination, retained more ChAT-positive neurons and performed better on a delayed match-to-position task than control animals. The data strengthen the theory that exogenous neurotrophic factors ameliorate the effects of Alzheimer's disease.

Neurotrophin receptor p75 mediates the uptake of the amyloid beta (Abeta) peptide, guiding it to lysosomes for degradation in basal forebrain cholinergic neurons.

Ovsepian SV, Antyborzec I, O'Leary VB, Zaborszky L, Herms J, Oliver Dolly J.

Brain Struct Funct Epub2013.

Accumulation of β-amyloid in the brain is considered one of the main causes of Alzheimer's disease. The increase in β-amyloid is accompanied by a reduction in levels of the high affinity nerve growth factor receptor (trkA) and cognitive impairment. The authors looked at levels of the low affinity nerve growth factor receptor (p75) that do not decline. Using a 0.8-µg injection of 192-Cy3 (Cat. #FL-01) into the medial prefrontal cortex of rats the authors assessed the transport of p75 and β-amyloid by microscopy. The results indicate that the primary destinations of both p75 and β-amyloid were the late endosome and lysosome.

P2Y1 receptors expressed by C1 neurons determine peripheral chemoreceptor modulation of breathing, sympathetic activity, and blood pressure.

Wenker IC, Sobrinho CR, Takakura AC, Mulkey DK, Moreira TS.

Hypertension 62(2):263-273, 2013.

Peripheral chemoreceptor activation response is mediated by catecholaminergic C1 cells in the rostral ventrolateral medulla (RVLM). The authors investigated the molecular mechanisms linking this drive to increased sympathetic activity and hypertension through a variety of methods, including lesioning C1 cells in the RVLM. Rats received 4.2-ng bilateral injections of Anti-DBH-SAP (Cat. #IT-03) into the RVLM. Comparison of lesioned animals to controls demonstrated that P2Y1 receptors on C1 cells in the RVLM are key components in the regulation of breathing, sympathetic nerve activity, and blood pressure.

GABAergic Terminals Are a Source of Galanin to Modulate Cholinergic Neuron Development in the Neonatal Forebrain.

Keimpema E, Zheng K, Barde SS, Berghuis P, Dobszay MB, Schnell R, Mulder J, Luiten PG, Xu ZD, Runesson J, Langel U, Lu B, Hokfelt T, Harkany T.

Cereb Cortex Epub2013.

In this work the authors sought to clarify the role of galanin during brain development. Several different techniques were used including the use of Galanin-SAP (Cat. #IT-34) on primary cell cultures from the fetal forebrains of rats. Cultured basal forebrain neurons were exposed to 5 ng/ml of Galanin-SAP for 8 hours, and cell death was assessed after 72 hours. Cholinergic cells were killed by Galanin-SAP, indicating that these neurons can use extracellular galanin-2 receptors to facilitate development.

Medial Septal Cholinergic Neurons Modulate Isoflurane Anesthesia.

Tai SK, Ma J, Leung LS.

Anesthesiology Epub2013.

General anesthesia is associated with a decrease in cholinergic function. This work examines the effect of volatile anesthetics such as isoflurane or ketamine in the context of cholinergic depletion. Rats received 105-ng bilateral injections of 192-IgG-SAP (Cat. #IT-01) into the medial septum. Anesthetic effects were evaluated using a loss of righting reflex test. There was no difference between lesioned and control groups in the response to ketamine. When treated with isoflurane, lesioned animals were affected for longer periods of time, and hippocampal response was reduced. The results suggest a role for septal cholinergic neurons in the sensitivity to isoflurane.

Epitopes of the Highly Immunogenic Trichomonas vaginalis alpha-Actinin Are Serodiagnostic Targets for Both Women and Men.

Neace CJ, Alderete JF.

J Clin Microbiol 51(8):2483-2490, 2013.

Trichomonas vaginalis is an anaerobic protozoan that is the most common nonviral causative agent for sexually-transmitted infections. The presence of T. vaginalis in men is usually asymptomatic, making it difficult to assess exposure to the organism. The authors examined sera from exposed individuals for reactivity to specific epitopes of trichomonad α-actinin. A recombinant version of trichonomad α-actinin was constructed and detected using Anti-6His (Cat. #AB-213). Some epitopes were reactive with sera from both men and women, making them potential diagnostic targets.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Leptin-sensitive neurons in the arcuate nucleus integrate activity and temperature circadian rhythms and anticipatory responses to food restriction.

Wiater MF, Li AJ, Dinh TT, Jansen HT, Ritter S.

Am J Physiol Regul Integr Comp Physiol Epub2013.

The arcuate nucleus (Arc) of the hypothalamus is known to participate in the regulation of feeding, adiposity, and leptin-dependent metabolism. The authors examined the role of leptin-receptive neurons in locomotor and temperature rhythms. Rats received four bilateral injections of Leptin-SAP (Cat. #IT-47) into the Arc; Blank-SAP (Cat. #IT-21) was used as a control. The lesion affected learning connected to light cycles, but not learning connected to food schedules, suggesting a mechanism for internal desynchrony that might play a role in obesity and other metabolic disorders.

C1 neurons: the body's EMTs.

Guyenet PG, Stornetta RL, Bochorishvili G, Depuy SD, Burke PG, Abbott SB.

Am J Physiol Regul Integr Comp Physiol 305(3):R187-204, 2013.

Although mainly known for their involvement in the control of arterial pressure, C1 neurons are also suspected to participate in numerous other physiological processes such as neuroendocrine response, glucose homeostasis, food consumption, and others. This review discusses the role of these neurons as 'emergency medical technicians' – cells that produce and modulate physiological survival responses to acute physical stress. The use of Anti-DBH-SAP (Cat. #IT-03) to delineate C1 neurons in the rostral ventrolateral aspect of the medulla oblongata is discussed.

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

Loss of neurons in rostral ventromedial medulla that express neurokinin-1 receptors decreases the development of hyperalgesia.

Khasabov SG, Simone DA.

Neuroscience 250C:151-165, 2013.

Previous data has indicated that neurokinin-1 receptors are located on ON cells in the rostral ventromedial medulla (RVM). ON cells are considered pronociceptive because noxious stimulation is stimulatory. In this work the authors eliminated ON cells using 0.3-µl injections of 1 µM SSP-SAP (Cat. #IT-11) into the left and right side of the RVM. Blank-SAP (Cat. #IT-21) was used as a control. SSP-SAP treatment did not change mechanical or heat withdrawal responses, or change morphine-induced analgesia. A significant reduction in the duration of nocifensive behaviors induced by various hyperalgesic stimulators indicated that these neurons are involved in pain facilitation rather than modulation.

Selective Immunotoxic Lesions of Basal Forebrain Cholinergic Neurons: Twenty Years of Research and New Directions.

Baxter MG, Bucci DJ.

Behav Neurosci Epub2013.

This review covers twenty years of basal forebrain cholinergic lesioning. The initial use of 192-IgG-SAP (Cat. #IT-01) is discussed, as well as other immunotoxins such as GAT-1-SAP (Cat. #IT-32) and OX7-SAP (Cat. #IT-02). The findings generated by the use of 192-IgG-SAP and how those data have helped forward the understanding of how the cholinergic system functions in the basal forebrain are detailed. The authors also discuss new directions in the field.

Noggin and Sonic hedgehog are involved in compensatory changes within the motoneuron-depleted mouse spinal cord.

Gulino R, Gulisano M.

J Neurol Sci 332(1-2):102-109, 2013.

Noggin (NOG) and Sonic hedgehog (Shh) are both involved in the generation and organization of neural tissues. In order to clarify the role of these two proteins in the regulation of neurogenesis and/or neuroplasticity the authors used a motoneuron depletion model in the mouse spinal cord. 3 µg of CTB-SAP (Cat. #IT-14) was injected into each of the medial and lateral gastrocnemius muscles and the expression of NOG and Shh were monitored. Motor performance also correlated with NOG and Shh levels, indicating that these proteins could play roles in regeneration and functional restoration.

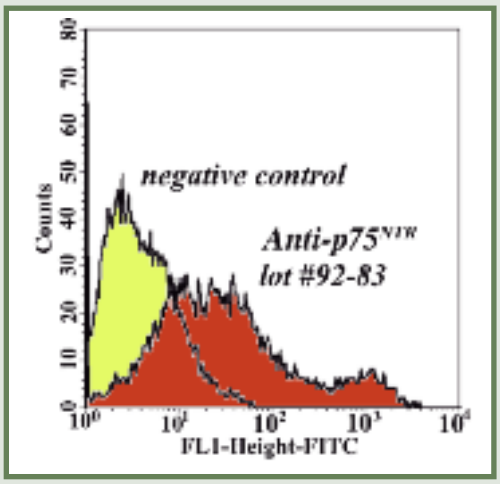
Cortical Metabolic Deficits in a Rat Model of Cholinergic Basal Forebrain Degeneration.

Gelfo F, Petrosini L, Graziano A, De Bartolo P, Burello L, Vitale E, Polverino A, Iuliano A, Sorrentino G, Mandolesi L.

Neurochem Res Epub2013.

In this work the authors investigated the connection between cholinergic depletion caused by conditions such as Alzheimer's disease and cerebral energy metabolism deficits. Rats received a 0.4-µg injection of 192-IgG-SAP (Cat. #IT-01) into the nucleus basalis magnocellularis. Neuronal metabolic activity was measured by assaying cytochrome oxidase (CO) activity. The unilateral injection produced a bilateral deficit in CO activity throughout the cortex, and the front and parietal cortices showed CO deficits before the lesion was complete. The data suggest a link between cholinergic hypofunctionality and metabolic deficit.

Targeting Talk: Product Questions



Q: We have a publication in review and put this statement in the paper, "The mouse monoclonal antibody to the low affinity nerve growth factor receptor (p75^{NTR}; Advanced Targeting Systems) was derived from immunization of mice with WM245 melanoma cells and recognizes p75^{NTR} in human, primate, rabbit, sheep, dog, cat, and pig. According to the manufacturer's information, the antibody was tested by flow cytometry." One of the reviewers wants to know more about the flow cytometry used to characterize this antibody (Cat. #AB-N07). Can you help, please?

A: This antibody is routinely tested by flow cytometry. The quality control flow data can be found on the data sheet on our website. HS294T cells, human metastatic melanoma cells, were used in flow cytometry with Anti-p75^{NTR} (ME20.4, Cat. #AB-N07). Cells were treated with 4 µg of AB-N07 and subsequently with Anti-murine IgG-FITC (Cat. #FL-07). This assay shows the binding affinity of AB-N07 to cells known to express p75^{NTR}.

Q&A Products

anti-p75^{NTR} (AB-N07)

Lauric Acid Polyclonal, conjugated (AB-T183)

Q: Does AB-T183 (Lauric Acid Rat Polyclonal, Conjugated) recognize lauric acid alone, or does it need to be conjugated to something (a protein carrier)?

A: This antibody targets conjugated Lauric Acid. It does not recognize free lauric acid. Antisera was preabsorbed on protein carriers and ammonium sulfate-purified. Using a conjugate Lauric acid-Gluteraldehyde-Protein Carrier (PC), antibody specificity was performed with an ELISA test by competition experiments with the following compounds:

Compounds	Cross-Reactivity Ratios
Lauric acid-PC	1
Caprylic acid-PC	1/300
Myristic acid-PC	1/400
Palmitic acid-PC	1/>50,000
Caproic acid-PC	1/>50,000
Oleic acid-PC	1/>50,000

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

Usage: Applications include immunohistochemistry (1/500-1/2,000) and immunocytochemistry.
Controls: Lauric Acid, conjugated, Cat. #AG-183

Targeting Topics: Recent Scientific References

(continued from page 4)

Implication of Cerebral Dopamine-beta Hydroxylase for Cardiovascular and Mood Regulation in Rats.

Chang ST, Liu YP, Huang CL, Wang PY, Tung CS.

Chin J Physiol 56(4)2013.

The ascending fibers affected by norepinephrine are involved in a variety of processes, including emotion, anxiety, and regulation of central autonomic outflows such as cardiovascular regulation and energy balance. The authors examined whether the loss of norephinephrine would cause autonomic failure in cardiovascular regulation. Rats received a single intraventricular injection of anti-DBH-SAP (Cat. #IT-03). Saporin (Cat. #PR-01) was used as a control. The results demonstrate that norepinephrine deficits in the brain influence reduction of excitatory responses to orthostatic stress.

Suggest It . . .

Do you have an idea for a new target? Contact us with your suggestion. If your target is chosen for development of a targeted toxin, we will provide the conjugate to you at no charge.

CONTACT US FOR MORE INFO: ATS@ATSBIO.COM

. . . Test It!

Role of spinal microglia in the development of morphine-induced hyperalgesia

(continued from page 1)

macrophage-1 antigen (Mac-1, 16–32 µg; Mac-1-SAP rat, Cat #IT-33) to selectively ablate spinal microglia in rats with established morphine-induced hyperalgesia. Injections of Mac-1-SAP or saporin alone as control (SAP, 20 µg; Cat #PR-01) were initiated after seven days of morphine treatment and performed at lumbar level once a day for three days. Mac-1-SAP significantly reduced the level of CD11b expression in the lumbar spinal dorsal horn (see Fig.1) and the treatment reversed mechanical and thermal hypersensitivity induced by morphine. Conversely, the immunotoxin did not affect the development of morphine tolerance in the same animals.

These findings point out the specific role of microglia in the development of pain hypersensitivity following morphine treatment. Thereafter, we dissected the underlying molecular mechanisms and found that morphine induced P2X4 receptor upregulation in spinal microglia, which in turn triggered the synthesis and release of brain-derived neurotrophic factor (BDNF). Microglial BDNF has been shown to induce pain hypersensitivity in spinal neurons by hampering the function of the K^+-Cl^- co-transporter KCC2, the main neuronal chloride extruding transporter in neurons, via trkB signaling.[5] Consistently, we found that such BDNF-trkB-KCC2 signaling cascade is activated by morphine and alters chloride-mediated inhibition on spinal neurons, thus increasing network excitability.

All together, our study indicates that morphine-induced hyperalgesia, as neuropathic pain, is a pathological alteration of pain sensitivity whose expression is gated by spinal microglia. Targeting at any level, this microglia-to-neuron cascade is a valuable strategy to improve the use of morphine in chronic pain.

References

1. Lee M., Silverman SM, Hansen H, Patel VB, Manchikanti, L. (2011) A comprehensive review of opioid-induced hyperalgesia. *Pain Physician* 14:145–161.
2. Crofford LJ. (2010) Adverse effects of chronic opioid therapy for chronic musculoskeletal pain. *Nat Rev Rheumatol* 6:191-7.
3. Vanderah TW, Suenaga NM, Ossipov MH, Malan TP Jr, Lai J, Porreca F. (2001) Tonic descending facilitation from the rostral ventromedial medulla mediates opioid-induced abnormal pain and antinociceptive tolerance. *J Neurosci* 21:279–286.
4. Ferrini F, Trang T, Mattioli TA, Laffray S, Del'Guidice T, Lorenzo LE, Castonguay A, Doyon N, Zhang W, Godin AG, Mohr D, Beggs S, Vandal K, Beaulieu JM, Cahill CM, Salter MW, De Koninck Y. (2013) Morphine hyperalgesia gated through microglia-mediated disruption of neuronal Cl^- homeostasis. *Nat Neurosci* 16:183-92.
5. Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y. (2005) BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 438:1017-1021.
6. Yoon SY, Patel D, Dougherty PM. (2012) Minocycline blocks lipopolysaccharide induced hyperalgesia by suppression of microglia but not astrocytes. *Neuroscience* 221:214-24.
7. González JC, Egea J, Del Carmen Godino M, Fernandez-Gomez FJ, Sánchez-Prieto J, Gandía L, García AG, Jordán J, Hernández-Guijo JM. (2007) Neuroprotectant minocycline depresses glutamatergic neurotransmission and Ca(2+) signalling in hippocampal neurons. *Eur J Neurosci* 26:2481-95.

Society for Neuroscience
November 10-13, 2013
San Diego, CA
Booth #1120

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

Upcoming Events

Targeting Teaser Solution

Congratulations to the puzzle solvers from last quarter. Each winner received a Knockout Mouse Tote Bag.

WINNERS: Maria Kot, Inst of Pharmacology, PAS, Krakow, Poland; Rene Schweickhardt, EMD Serono, Billerica, MA; Prasanthi Geda, Merck, Boston, MA; Shunsuke Takasuga, Akita University, Akita-shi, Japan; Judene Bliss, Roswell Park Cancer Inst, Buffalo, NY; Terry Beltz, Univ Iowa, Iowa City, IA; Richard Fuerstenberg, R&D Systems, Minneapolis, MN; Jheem Medh, California State Univ, Northridge, CA; Adam Farmer, Triangle Research Labs, Research Triangle Park, NC; Shelle Malkmus, University of California, San Diego, CA; Sherie Ma, Florey Inst of Neuroscience and Mental Health, Parkville, Australia; Clay Archer, University of California, San Diego, CA; Glenn Kageyama, Cal Poly University, Pomona, CA; Bob Speth, Nova Southeastern Univ, Fort Lauderdale, FL

Bob Speth with his prize tote bag.
Photo courtesy of Eduardo Carrera.

The solution to the puzzle was:

Jumbles:
PLURIPOTENT
HEDGEHOG
DORSAL
GANGLIONIC
EMBRYONIC

What the ATS Knockout Mouse did to support himself while earning his PhD in Neuroscience.

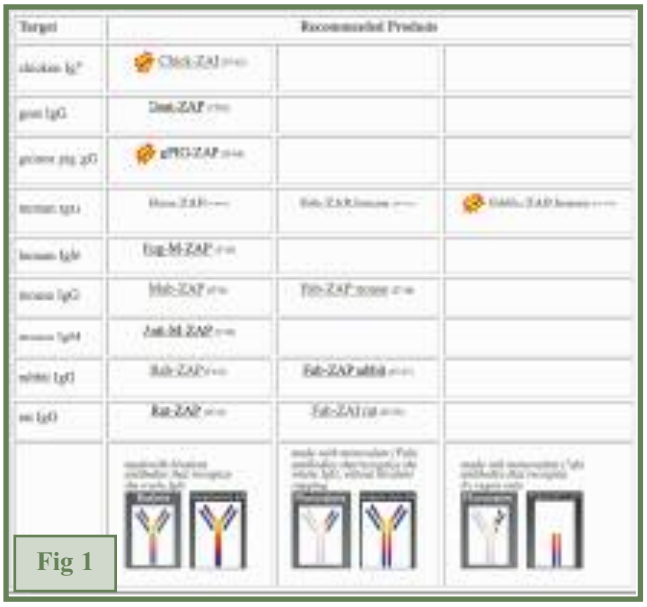
Answer:
HE WORKED AS A ... MODEL.

Solve this quarter's teaser at www.ATSbio.com/news/13q4_teaser.html.

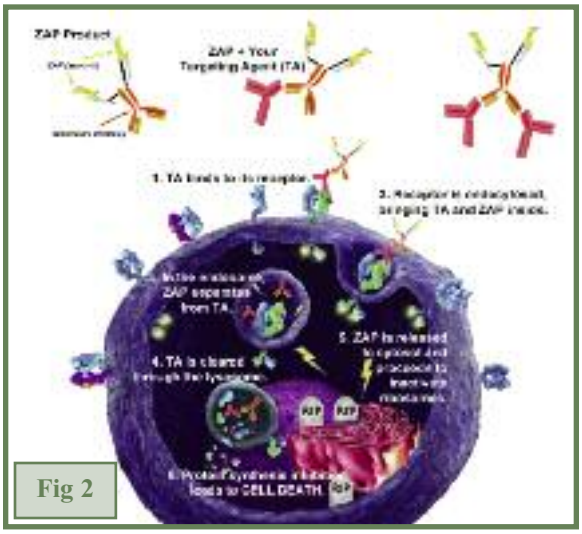
Targeting Tools: Featured Products

Contributed by Brian Russell, Product Manager

ZAP Antibody Internalization Kit (Cat. #KIT-100)



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. Targeted conjugates are widely used to escort payloads to specific cell populations *in vitro* and *in vivo* for both basic research and pharmaceutical development. The development of an effective and specific targeted conjugate is a long and costly process. A molecule that targets the marker of choice (a Targeting Agent) must be identified and produced; internalization and specificity must be verified and characterized. Desirable traits of a Targeting Agent (TA) include high specificity and rapid internalization. The TA can be an antibody, peptide, protein, or any other molecule that recognizes a cell-surface marker. Antibodies often make the best targeting agents, and the choice of the correct antibody is crucial to the specificity and performance of payload delivery.



The ZAP products produced by Advanced Targeting Systems, Inc. allow a large number of antibodies to be screened quickly and cost-effectively for specificity, functional binding, internalization, and EC50 determination. The wide selection of these secondary conjugates identified by the ZAP moniker provide ideal tools for screening potential antibodies (Fig 1). They are constructed using either species-specific polyclonal anti-IgG antibodies, or streptavidin (alternate kit for use with biotinylated targeting agents), that are chemically attached to Saporin, the most potent of the plant ribosome-inactivating proteins. The mechanism of action of ZAP-based conjugates is detailed in Fig 2.

Use of a ZAP conjugate eliminates the time-consuming and expensive step of conjugating each TA candidate to the payload: the ZAP product can simply be added to cells together with the TA candidate. Once the materials have been administered, the antibody 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. Cells not expressing the target do not bind or internalize the ZAP-antibody complex, and are not affected. Saporin has no binding chain, and no means of getting into cells on its own.

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 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 EC50 useful in determining if the candidate-antibody should be pursued at the next level.

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

10451 Roselle Street #300
San Diego, CA 92121

PRESORTED STD.
U.S. POSTAGE
PAID
SAN DIEGO, CA
PERMIT # 2686

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.

UNSCORENIECE

PROMINHE

FARCEKIB

STORRENTRAP

HERICOLD

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

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

ANSWER: IF I ONLY HAD . . . ◯ ◯◯◯◯◯ !

WIN!

SOLVE the puzzle online with the correct solution by December 31, 2013.

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

www.atsbio.com/news/13q4_teaser.html