Oct-Nov-Dec 2013
Volume 14, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

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

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

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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)*

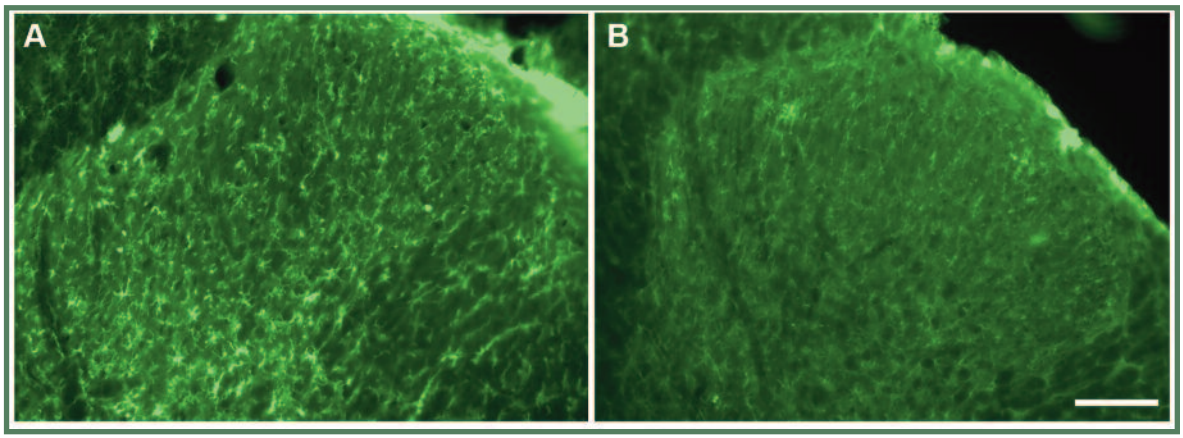


Figure 1. Microglia depletion in the lumbar region of the spinal cord after intrathecal injections of Mac-1-SAP. Microglia are immunohistochemically identified by a mouse CD11b antibody, clone OX-42 (1:500). A) A representative cervical spinal dorsal horn section obtained from a rat which was subcutaneously 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.

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.

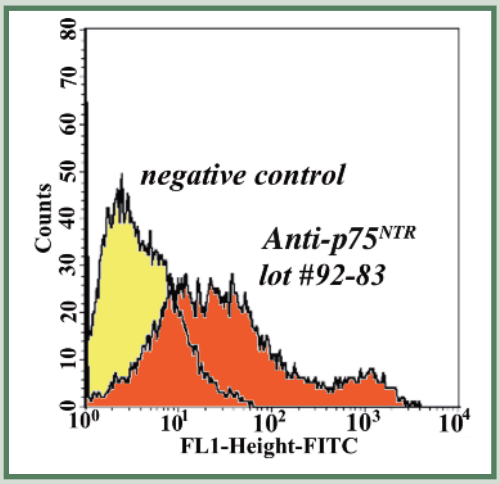
Cortical Metabolic Deficits in a Rat Model of Cholinergic Basal Forebrain Degeneration.

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.

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. . . 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

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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 showing off his prize.
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.

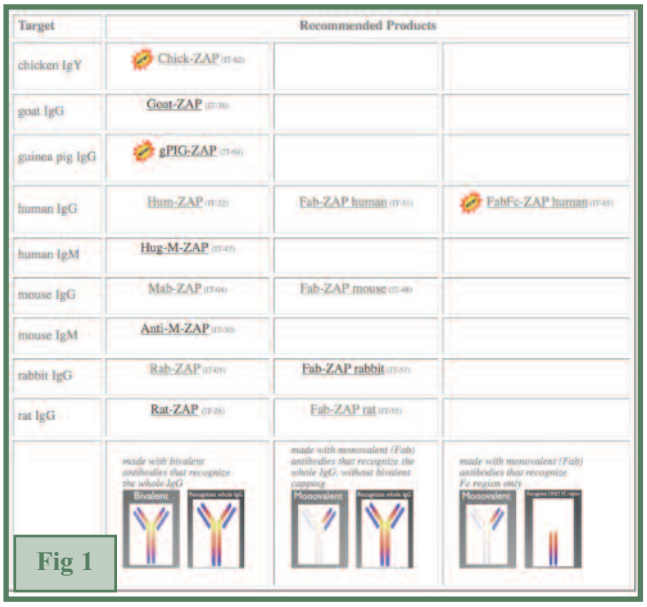


Fig 1

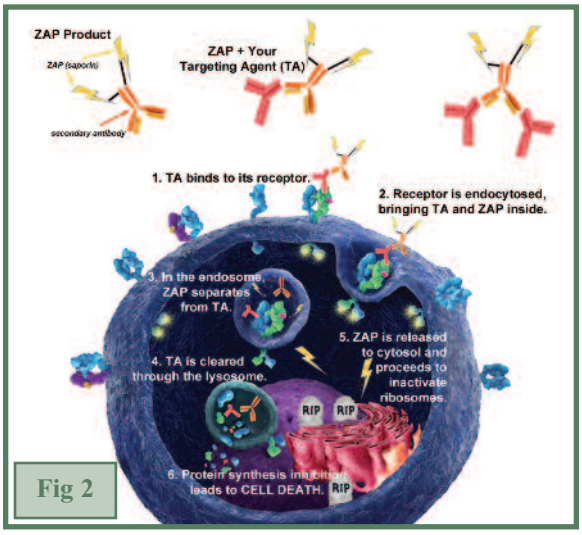
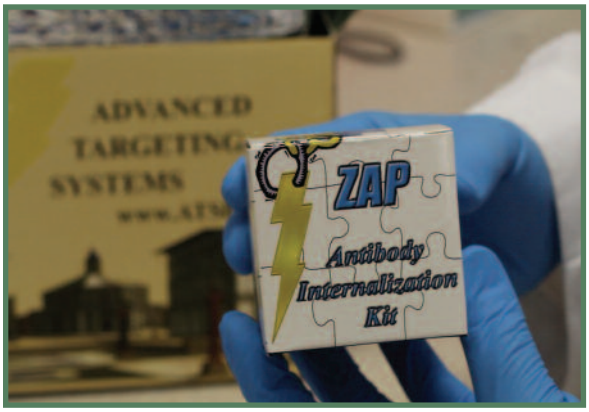


Fig 2

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.



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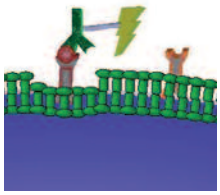
§ or anything recognized on the cell surface and internalized.

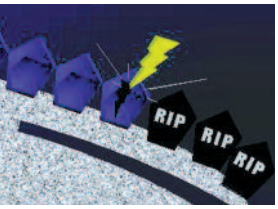
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



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

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



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

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

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