Targeting Trends

Reporting the latest news in Molecular Surgery

Celebrating Twenty Years of Science

Advanced Targeting Systems (founded 22 Apr 1994)

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

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

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

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

Photo Credit: Z.F. Chen et al.
Celebrating Twenty Years of People Who Make ATS Special

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

The 3 Guys of Piero della Francesca

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

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

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

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

Targeting Teaser Solution

The solution to the puzzle was:
Jumbles: PHARMACEUTICAL  INTRATHECAL  BROWN  PATHOLOGY  VIDEOGRAPHY

What the scientist said about studying gravity.
Answer: Just don’t let it... GET YOU DOWN.

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


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

Congratulations to the puzzle solvers from last quarter. Each winner has received a tote bag featuring the 25th Annual SBC.
Targeting Topics: Top 20 Most-Cited References


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


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


NOTE: In order to make space for the 20th Anniversary special additions, this quarter’s references have abbreviated summaries beginning on Page 4. Please visit www.ATSBio.com/news/14q2_news.html to see the complete summaries.
Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Habenerian kisspeptin modulates fear in the zebrafish.
Ogawa S, Nathan FM, Parhar IS.


Zebrafish express kiss1 mRNA which is a conserved ortholog of the mammalian KISS1/Kiss1 making zebrafish a viable model for investigating the role of kisspeptin in various brain systems. Animals received 1 μg of the custom conjugate kiss-SAP via an intracranial injection. Blank-SAP (Cat. #IT-21) was used as a control.

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


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

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

Roland JJ, Stewart AL, Janke KL, Gielow MR, Kostek JA, Savage LM, Servatius RJ, Pang KC.


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

Cholinergic contributions to supramodal attentional processes in mice.

Ljubojevic V, Luu P, De Rosa E.


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

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

Quadros EV, Nakayama Y, Sequeira JM.


The authors used a custom conjugate of antibodies generated against the TCB1R and saporin to eliminate cancer cell lines in culture, applying the conjugate to cells in a dosing range of 0.156-5 nM, 2.5 nM to have the optimal effect.

Neutralization of Plasmodium falciparum Merozoites by Antibodies against PFRH5.


PFRH5 binds basigin on erythrocytes, and through the use of anti-basigin (Cat. #AB-42), among other antibodies, the authors better characterized aspects of this binding that may be useful in preventing malaria.

The novel EpCAM-targeting monoclonal antibody 3-171 linked to saporin is highly cytotoxic after photochemical internalization in breast, pancreas and colon cancer cells lines.

Lund K, Bostad M, Skarpén E, Braunagel M, Krauss S, Duncan A, Hogset A, Selbo P.

MAbs 6(3)2014.

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

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

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

Cell Transplant Epub2014.

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

Neuroprotective effects of donepezil against cholinergic depletion.


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

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

Suto T, Severino AL, Eisenach JC, Hayashida KI.

Neuropharmacology 81C:95-100, 2014.

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

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

Li AJ, Wang Q, Ding TT, Powers BR, Ritter S.


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

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

Weishaar CL, Winkelstein BA.

J Pain Epub2014.

The authors examined the role of NK1-expressing spinal cells in this pathway. Rats received 100 ng SSP-SAP (Cat. #IT-11) via
Q: Your recent issue of Targeting Trends stated that it was unlikely that saporin compounds or constituents would be excreted in urine or feces. However, you acknowledge that experimental data is lacking. Have there been any tests of animal urine or feces for saporin content? My animal care staff are concerned.

A: One of the reasons that no studies have been done on excretion of saporin is that there isn't much on the theoretical side to cause concern. The primary issue is that the quantity used in mice (and even rabbits) is so small that when looked at in human terms (i.e., an animal 10 to 100-times larger), the dosage becomes insignificant. The LD₅₀ for saporin in mice is 4-8 mg/kg; that would translate in humans to more than you'll ever use! The immunotoxins, which contain only about 20% saporin by weight, really do not contain all that much saporin.

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

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

Reference

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

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

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

2000
Robert Sloviter and Jennifer Martin used SSP-SAP (Cat# IT-11)

2001
Mary Ann Greco used Orexin-SAP

2002
Lique Coolen and William Truitt used SSP-SAP (Cat# IT-11)

2003
Jill McGaughy used Anti-DBH-SAP (Cat# IT-03)

2004
Michelle Pearson used IB4-SAP (Cat# IT-10)

2005
W. Zhang used Dermorphin-SAP (Cat. #IT-12) and CCK-SAP (Cat. #IT-26)

2006
Neelima Chauhan used mu p75-SAP (Cat# IT-16)

2007
Kevin Pang used Anti-GAT-SAP (Cat# IT-32)

2008
Arshad Khan used Anti-DBH-SAP (Cat# IT-03)

2009
Ai-Jun Li used Leptin-SAP (Cat# IT-47) and Blank-SAP (CAT #IT-21)

2010
Yan Liu used mu p75-SAP (Cat# IT-16)

2011
Melinda Smith and Sally Krajewski used NK3-SAP (Cat# IT-63)

2012
Ko Zushida used Bombesin-SAP (Cat# IT-40)

2013
Damla Khan used Mac-1-SAP (rat) (Cat# IT-33)
Targeting Tools: Top Twenty (Five Each in Four Categories)

Top Five Targeted Toxins

#1. 192-IgG-SAP (192-Saporin)  (Cat. #IT-01)
#2. Anti-DBH-SAP  (Cat. #IT-03)
#3. mu p75-SAP  (Cat. #IT-16)
#4. IB4-SAP  (Cat. #IT-10)
#5. Mac-1-SAP mouse/human  (Cat. #IT-06)

TOP TARGETED TOXIN OF 2013
192-IgG-SAP (192-Saporin)  (Cat. #IT-01)

Top Five Antibodies

#1. NGFr (mu p75) Rabbit Polyclonal  (Cat. #AB-N01)
#2. NGFr (ME20.4, p75) Mouse Monoclonal  (Cat. #AB-N07)
#3. trkA Rabbit Polyclonal  (Cat. #AB-N03)
#4. NGFr (mu p75) Rabbit Polyclonal, affinity-purified  (Cat. #AB-N01AP)
#5. Angiotensin II receptor (AT-2r) Rabbit Polyclonal, affinity-purified  (Cat. #AB-N28AP)

TOP ANTIBODY OF 2013
NGFr (ME20.4, p75) Mouse Monoclonal  (Cat. #AB-N07)

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

Contributed by Lisa E. Goehler and Ronald P.A. Gaykema

Top Five Secondary Conjugates

#1. Mab-ZAP  (Cat. #IT-04)
#2. Streptavidin-ZAP  (Cat. #IT-27)
#3. Anti-6 His-ZAP  (Cat. #IT-52)
#4. Rab-ZAP  (Cat. #IT-05)
#5. Fab-ZAP rat  (Cat. #IT-55)

TOP SECONDARY CONJUGATE OF 2013
Fab-ZAP human  (Cat. #IT-51)

Top Five in Publications

#1. 192-IgG-SAP (192-Saporin)  (Cat. #IT-01)
#2. Anti-DBH-SAP  (Cat. #IT-03)
#3. SSP-SAP  (Cat. #IT-11)
#4. mu p75-SAP  (Cat. #IT-16)
#5. Streptavidin-ZAP  (Cat. #IT-27)
“Molecular Surgery for Scientists”

www.ATSbio.com

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

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...

Targeting Teaser

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

NEXTOUNIRO

ZANYALE

SKROWER

SNOBBEMI

JOINTINCE

What do you wish someone for their 20th anniversary?

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

ANSWER:

MAY YOU ENJOY ...

WIN!

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

WIN a large, reusable flat-bottom tote bag celebrating ATS’s 20 Years of Targeting Excellence!

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