

# Targeting Trends

Reporting the latest news in Molecular Surgery

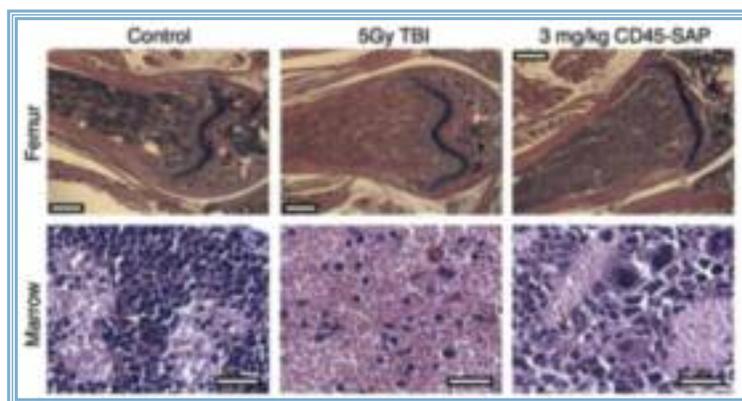
## Targeted depletion of hematopoietic stem cells promises safer transplantation

By Rahul Palchaudhuri, Ph.D., Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, Massachusetts, USA

Hematopoietic stem cell transplantation (HSCT) has been clinically used for 58 years and offers life-saving therapies for a variety of malignant and non-malignant blood disorders. Currently 50,000 transplants are performed globally per year with 90% of these for the treatment of malignancies.

Prior to receiving a transplant, a patient must be “conditioned” which serves to destroy resident hematopoietic stem cells in the marrow, in order to create niche vacancies for successful donor stem cell engraftment. Unfortunately, current conditioning strategies are non-targeted and genotoxic as they use DNA-damaging whole body irradiation and chemotherapy. As expected, these crude methods induce severe short-term and long-term conditioning-related toxicities that ultimately limit the application of hematopoietic stem cell transplantation, particularly in non-malignant conditions (e.g. sickle cell anemia, thalassemia, immunodeficiencies and autoimmune conditions).

While antibodies are potentially an appealing alternative to current conditioning methods, previous antibody-based strategies relying on naked antibodies have been met with limited success in immunocompetent animals. We therefore explored antibody-based immunotoxins created using the ribosome-inactivating protein, saporin, as a means of depleting hematopoietic stem cells in immunocompetent mice. By combining various biotinylated monoclonal antibodies with streptavidin attached to saporin (Streptavidin-ZAP, Cat. #IT-27), we created immunotoxins and screened their ability to achieve stem cell depletion *in vivo*. From our screen, we identified CD45-SAP as a potent stem cell-depleting agent capable of depleting >98% of hematopoietic stem cells following a single-dose administration. Using CD45-SAP we demonstrated successful donor stem cell engraftment with long-term donor chimerism levels greater than 90%.



**Fig. 1:** Hematoxylin and eosin staining of femur marrow sections of non-treated control, 3 mg/kg CD45-SAP or 5Gy TBI conditioned C57BL/6 mice 2 days post-conditioning. Representative images from independent experiments ( $n = 2$  mice/group) are shown. Scale bars in top and bottom images represent 500  $\mu\text{m}$  and 20  $\mu\text{m}$ , respectively.<sup>1</sup>

(continued on page 6)

 Brian Russell - Editor	<b>Letter from the President:</b> “What’s ZAP?” Page 2	<b>New Products &amp; Tools:</b> Orexin/Hypocretin Products Page 3	<b>Journal Time:</b> Latest Pubs & Refs reviewed Pages 4-5	<b>Talking About Targeting:</b> Streptavidin-ZAP Page 7
---	--	--	--	---

# What's ZAP?

Denise Higgins - President



Fig. 1 Saporin is obtained from the seeds of the Soapwort plant (*Saponaria officinalis*). Saporin is a plant enzyme with N-glycosidase activity that deurinates a specific nucleotide in the ribosomal RNA 28S, thus irreversibly blocking protein synthesis. It belongs to the well-characterized family of ribosome-inactivating proteins (RIPs).

Wuzzup? No. What's ZAP? Some of our products have SAP in the name, like 192-IgG-SAP (Cat. #IT-01). Some of our products have ZAP in the name, like Hum-ZAP (Cat. #IT-22).

First, what's the same about ZAP and SAP? They both mean Saporin. The payload that Advanced Targeting Systems made famous to specifically eliminate targeted cells. For those of you new to this technology, Saporin is a ribosome-inactivating protein (Fig. 1).

Now, what's different about ZAP and SAP? The difference is in what the conjugate can do. A SAP conjugate has two components: 1) Saporin and 2) A targeting agent that is recognized on the cell surface and internalized. A ZAP conjugate has two components: 1) Saporin and 2) A non-specific agent that is NOT recognized on the cell surface and internalized (e.g. a secondary antibody, nonspecific peptide, or streptavidin).

If you want to make a saporin conjugate with your cell surface targeting agent, check out our ZAP products: ZAP Internalization Kits (Z-Kits) and Streptavidin products (see Page 7 for more information).

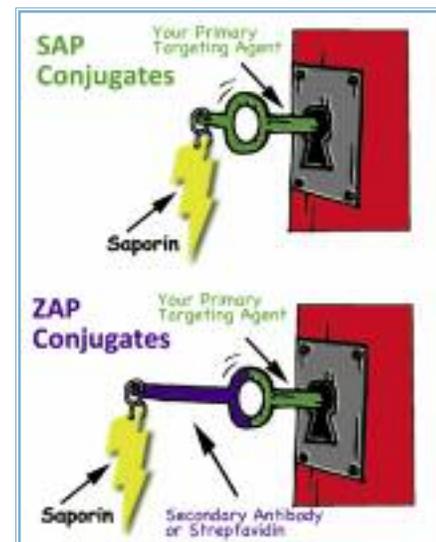


Fig. 2. The difference between SAP conjugates and ZAP conjugates. SAP conjugates target and eliminate specific cells. ZAP conjugates need a primary targeting agent to be internalized.

## Presidential Promotions for this Quarter



### Summer special on this year's HOT antibodies

Buy one, Get one free (equal or lower-priced item) — mix or match.

Enter coupon code **HOT1** at checkout. Offer expires July 31, 2016.

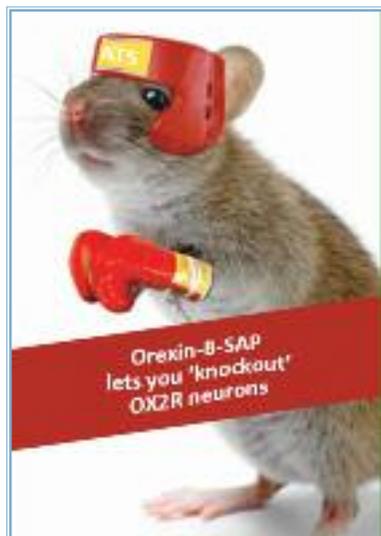
Choose from these top 20 best sellers:

Acrolein Rabbit Polyclonal, Conjugated [AB-T091]  
 Angiotensin II receptor (AT-1r) Rabbit Polyclonal, affinity-purified [AB-N27AP]  
 Angiotensin II receptor (AT-2r) Rabbit Polyclonal, affinity-purified [AB-N28AP]  
 Corticotropin Releasing Hormone Rabbit Polyclonal [AB-02]  
 Dopamine Rabbit Polyclonal, Conjugated [AB-T07]  
 Dopamine Transporter Rat Monoclonal (DAT-NT) [AB-N18]  
 Fibroblast Growth Factor Rabbit Polyclonal, mammalian [AB-07]  
 GABA Rabbit Polyclonal, Conjugated [AB-T10]  
 Histamine Rabbit Polyclonal, Conjugated [AB-T024]  
 Melatonin Polyclonal, Conjugated [AB-T177]

Metabotropic Glutamate Receptor 2 (mGluR2) Mouse Monoclonal [AB-N32]  
 NGFr (mu p75) Rabbit Polyclonal, affinity-purified [AB-N01AP]  
 NGFr (mu p75) Rabbit Polyclonal [AB-N01]  
 NGFr (ME20.4, p75) Mouse Monoclonal [AB-N07]  
 NO-L-Cysteine Mouse Monoclonal, Conjugated [AB-T125]  
 Quinolinic Acid Rabbit Polyclonal, Conjugated [AB-T095]  
 RFP Mouse Monoclonal [AB-332]  
 Trans-hydroxyproline Rabbit Polyclonal, Conjugated [AB-T044]  
 Tri-methyl Lysine Rabbit Polyclonal [AB-265]  
 vGAT Rabbit Polyclonal, affinity-purified [AB-N44AP]

# ATS Toolbox - Orexin Products

## Orexin-B-SAP (Cat. #IT-20)



**Fig. 1:** Targeted toxins offer the ability to develop “knockouts” through cell surface-based targeting that has several advantages over the gene-based approach. The “knockout” has a slight but important difference: instead of knocking out a particular protein from a set of cells (or even the whole animal), you eliminate a particular cell type. And this happens at your convenience: you inject the animal, put it back in its cage and then usually four days later, behavioral differences begin to show. These usually become permanent after a week or so.

ATS is pleased to re-release a product and kit specific for the Orexin 2 receptor (OX2R). The Orexin 1 and Orexin 2 receptors are found in the perifornical area/latero-posterior hypothalamus, and projections from this area cover much of the brain. These receptors have been implicated in various neurophysiological and neuropsychological disorders such as narcolepsy, insomnia, drug addiction, anxiety, and migraine headaches. The Orexin-B-SAP conjugate consists of the rat/mouse-specific orexin-B peptide conjugated to Saporin. Orexin-B binds to OX2R with approximately 5X greater affinity than to OX1R (Fig. 2).

Orexin-B-SAP (Cat. #IT-20) is highly specific for cells that express OX2R. Instead of spending precious time and money producing an OX2R knockout animal, you can use Orexin-B-SAP to specifically eliminate cells that express OX2R (Fig. 1). Orexin-B-SAP also allows you to study the behavioral effects before and after treatment and subsequent elimination of OX2R expressing cells. The specificity and efficacy of Orexin-B-SAP has recently been reported in the published work of Schwartz *et al.*<sup>1</sup>

1. Schwartz MD, Nguyen AT, Warriar DR, Palmerston JB, Thomas AM, Morairty SR, Neylan TC, Kilduff TS. (2016) Locus Coeruleus and Tuberonmammillary Nuclei Ablations Attenuate Hypocretin/Orexin Antagonist-Mediated REM Sleep. *eNeuro* 3(2) PMID: 27022631.

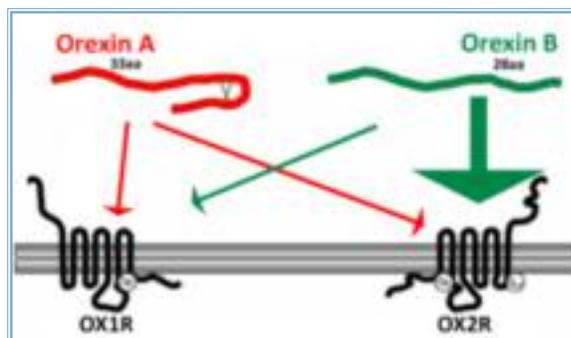
## Orexin Receptor Antibody Conjugates (Cat. #BETA-007 and BETA-008)

Anti-OX1R-SAP (Cat. #BETA-007) eliminates mouse, rat, and guinea pig cells expressing the orexin 1 receptor. All other cells are left untouched.

Anti-OX2R-SAP (Cat. #BETA-008) eliminates rat cells expressing the orexin 2 receptor (OX2R). All other cells are left untouched.

These two antibodies are being offered as part of our Beta Testing program. They have not been characterized or reported in scientific literature but they are being offered at no charge for the product (S&H extra). The researcher who first publishes data\* will receive a \$500 credit for use on ATS products.

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



**Fig. 2.** The orexin receptor (also referred to as the hypocretin receptor) is a G-protein-coupled receptor that binds the neuropeptide orexin. There are two variants, OX1R and OX2R. There are two orexin peptides: Orexin A and Orexin B. These peptides bind to both receptors, however, Orexin B shows a >5-fold selectivity for OX2R. Orexin A is equipotent at both receptors.

# Recent Publications & References

Reviewed by Brian Russell

## Involvement of nigral oxytocin in locomotor activity: A behavioral, immunohistochemical and lesion study in male rats.

Angioni L, Cocco C, Ferri GL, Argiolas A, Melis MR, Sanna F.

*Horm Behav* 83:23-38, 2016.

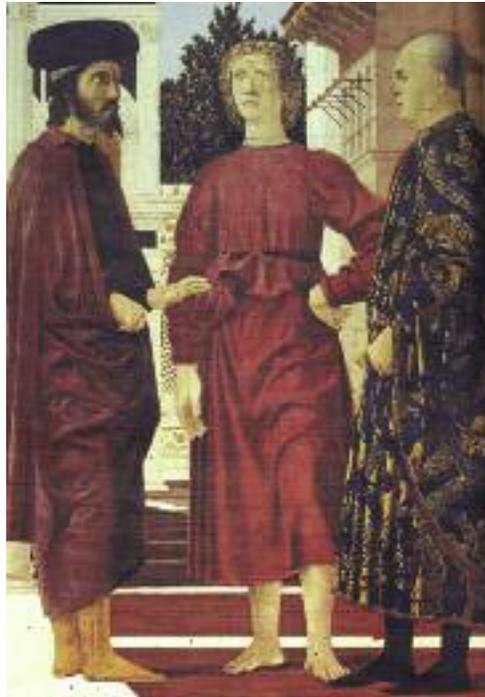
Oxytocin is well known for its hormonal role in lactation and parturition, but also exerts widespread actions in central nervous system. Previous experiments revealed the existence of a correlation between the changes in locomotor activity found in Oxytocin-SAP-treated rats and the extent of the changes in nigral TH and vesicular glutamate transporters immunoreactivity, provide support for a modulatory role of oxytocin on locomotor activity at the level of the substantia nigra. The day after a prior assessment of spontaneous locomotor activity, rats were randomly injected bilaterally with 0.3  $\mu$ L of Oxytocin-SAP (Cat. #IT-46, 60 ng/ $\mu$ L/site), or with the same amount of Blank-SAP (Cat. #IT-21, 60 ng/ $\mu$ L/site) or with vehicle (0.3  $\mu$ L/site of PBS, pH 7.4). Whether oxytocin may be considered as a target for controlling motor disturbances, as those occurring in Parkinson's disease and/or in other motor disturbances related to basal ganglia dysfunctions, remains to be evaluated.

## Gi-Protein Coupled 5-HT<sub>1B/D</sub> Receptor Agonist Sumatriptan Induces Type I Hyperalgesic Priming.

Araldi D, Ferrari LF, Levine JD.

*Pain* 2016.

The present study explored the possibility that, like MOR and A<sub>1</sub>-adenosine receptor agonists, triptans would also induce type II hyperalgesic priming. In addition, they explored the 5-HT receptor subtypes at which triptans act (5-HT<sub>1B</sub>, 5-HT<sub>1D</sub> and 5-HT<sub>7</sub>) to induce priming. They report that while sumatriptan, a prototypical 5-HT<sub>1B/D</sub> receptor agonist induces hyperalgesic priming, this priming meets the criteria for type I rather than type II priming. Isolectin B<sub>4</sub> (IB<sub>4</sub>)-saporin (Cat. #IT-10), was diluted in saline, and a dose of 3.2  $\mu$ g, in a volume of 20  $\mu$ L was administered intrathecally to rats. The neurotoxin [Sar<sup>9</sup>,Met<sup>(O<sub>2</sub>) 11]-substance</sup>



P-saporin (SSP-Saporin, Cat. #IT-11) was diluted in saline, and a dose of 100 ng, in a volume of 20  $\mu$ L was administered intrathecally. In a model of pain chronification, sumatriptan induces both mechanical hyperalgesia at the site of injection and type I hyperalgesic priming, in nociceptors innervating the cutaneous injection site.

## Retinal Waves Modulate an Intraretinal Circuit of Intrinsically Photosensitive Retinal Ganglion Cells.

Arroyo DA, Kirkby LA, Feller MB.

*J Neurosci* 36(26):6892-6905, 2016.

The researchers explore the neural circuits underlying the ipRGC driven light responses of the developing retina and the mechanisms by which retinal waves regulate these circuits. They demonstrate that, even in the presence of cholinergic waves, ipRGC gap junction microcircuits propagate light-driven signals, thus strongly contributing to the overall light response of the developing retina. Following fixation, retinas were washed in PBS and remounted onto a new piece of filter paper. They were incubated in blocking buffer and then in primary immunoreaction solution, 1:2500 rabbit anti-melanopsin (Cat. #AB-N38). Results show that, during development,

ipRGCs form extensive gap junction microcircuits that shape the early retinal light response. Retinal waves exert a far-reaching, neuromodulatory influence on these circuits via dopaminergic modulation of gap junctions, thus potentially impacting the processing of early visual input.

## Brainstem opioidergic system is involved in early response to experimental SAH.

Cetas JS, McFarlane R, Kronfeld K, Smitasin P, Liu JJ, Raskin JS.

*Transl Stroke Res* 6(2):140-147, 2015.

Subarachnoid hemorrhage (SAH) is a particular type of stroke that has high morbidity and mortality. The damage due to SAH is manifested in numerous ways, including global hypoperfusion, neuronal death, infarcts, microhemorrhages, and cortical spreading depression - as well as other acute autonomic dysfunctions. In this work the authors investigated how some autonomic and sensorimotor systems in the rostral ventromedial medulla (RVM) are involved in the maintenance of cerebral blood flow in a SAH model. Rats received 1 pmol total of dermorphin-SAP (Cat. #IT-12) in bilateral injections to the RVM. Blank-SAP (Cat. #IT-21) was used as a control. The results indicate that  $\mu$ -opioid receptor-expressing cells in the RVM are important in reducing mortality rates after SAH.

## Role of the RVM in Descending Pain Regulation Originating from the Cerebrospinal Fluid-Contacting Nucleus.

Fei Y, Wang X, Chen S, Zhou Q, Zhang C, Li Y, Sun L, Zhang L.

*Neurochem Res* 41(7):1651-1661, 2016.

The researchers investigated whether the CSF-contacting nucleus contributed to descending pain modulation in normal and neuropathic rats, and detected the 5-HT expression changes in both RVM and spinal dorsal cord. They also detected the possible anatomical and function correlation between the CSF-contacting nucleus

(continued on page 5)

# Recent Publications & References

(continued from page 4)

and the RVM. Targeted ablation of the CSF-contacting nucleus was performed using CTB-SAP (Cat. #IT-14; 500 ng/3 µl), which was administered i.c.v. to the normal rats and rats 7 days before the CCI procedure. Based on the findings of the present study, they believe that the CSF-contacting nucleus may act as a component of descending pain regulation system. RVM, which acts as an important brain nucleus, is involved in the relay of nociceptive information between the CSF-contacting nucleus and spinal cord. Moreover, RVM 5-HT system plays a critical role in descending pain inhibition originating from the CSF-contacting nucleus.

## Functional characterization of a mouse model for central post-stroke pain.

Gritsch S, Bali KK, Kuner R, Vardeh D. *Molecular Pain* 12:1744806916629049, 2016.

While clinical evidence has pointed toward central pain pathway dysfunction in central post-stroke pain (CPSP), the underlying mechanisms have not been defined. In this work the authors created a mouse model of CPSP through lesions of the thalamic ventral posterolateral nucleus. In order to examine the role of neurokinin-1 receptor-expressing (NK1R) neurons in lamina I/III of the spinal cord in the development and maintenance of CPSP the authors administered 1 µmol intrathecal injections of SSP-SAP (Cat. #IT-11). Saporin (Cat. #PR-01) was used as a control. While the NK1R+ neurons in the spinal cord were not involved in establishing CPSP, the data indicate that sensory changes in the mice are comparable to those observed in human patients with CPSP.

## Possible Involvement of the Rat Hypothalamo-Neurohypophysial-Spinal Oxytocinergic Pathways in Acute Nociceptive Responses.

Matsuura T, Kawasaki M, Hashimoto H, Yoshimura M, Motojima Y, Saito R, Ueno H, Maruyama T, Ishikura T, Sabanai K, Mori T, Ohnishi H, Onaka T,

Sakai A, Ueta Y. *J Neuroendocrinol* 28(6):2016.

It has been suggested that the amplification of GABAergic neurons in the inhibitory system induces the selective inhibition by Oxytocin (OXT) of excitability in the spinal cord, and the pain transmitted from the periphery to the dorsal horn of the spinal cord by this action may be attenuated at the spinal cord level. Rats were injected IT with Oxytocin-SAP (Cat. #IT-46) dissolved in saline (0.06 µg/µl), Blank-SAP (Cat. #IT-21) dissolved in saline (0.06 µg/µl), or saline. Formalin-induced acute nociception activated OXT-containing cells in both the magnocellular and parvocellular divisions of hypothalamus, and that the parvocellular division remains activated longer than the magnocellular division. Acute nociception-induced activation of the hypothalamo-neurohypophysial system caused elevation of plasma OXT levels. In addition, the OXTergic spinal pathway may be involved in pain modulation via OXTRs in the spinal cord.

## Effects of central administration of oxytocin-saporin cytotoxin on chronic inflammation and feeding/drinking behaviors in adjuvant arthritic rats.

Matsuura T, Kawasaki M, Hashimoto H, Yoshimura M, Motojima Y, Saito R, Ueno H, Maruyama T, Sabanai K, Mori T, Ohnishi H, Sakai A, Ueta Y. *Neurosci Lett* 621:104-110, 2016.

In the present study, Oxytocin-SAP, which chemically disrupts oxytocin (OXT) signaling was administered centrally and an OXT receptor (OXTR) antagonist administered peripherally to determine whether central and peripheral OXT is involved in chronic inflammation and feeding/drinking behavior in adjuvant arthritis (AA) rats. Rats were injected i.t. with Oxytocin-SAP (Cat. #IT-46) or Blank-SAP (Cat. #IT-21) dissolved in saline (0.06 µg/µl). The results demonstrated that the arthritis index values were significantly enhanced and suppression of food intake was transiently attenuated in Oxytocin-SAP treated rats when AA developed, The arthritis index and food

intake did not significantly change in the OXTR antagonist i.p.-injected rats. These results suggest that central oxytocinergic pathways may be involved in anti-inflammation at the spinal level and suppression of feeding behavior at the forebrain-brainstem level in AA rats.

## Ablation of KNDy neurons results in hypogonadotropic hypogonadism and amplifies the steroid-induced LH surge in female rats.

Mittelman-Smith MA, Krajewski-Hall SJ, McMullen NT, Rance NE. *Endocrinology* 157(5):2015-2027, 2016.

KNDy neurons are a subpopulation of neurons in the infundibular nucleus that coexpress estrogen receptor  $\alpha$ , kisspeptin, and neurokinin B (NKB) mRNA. Previous work indicated that altered signaling from KNDy neurons may play a role in the low levels of circulating sex steroids found in hypogonadotropic hypogonadism. Rats received bilateral 10-ng injections of NK3-SAP (Cat. #IT-63) dorsal to the arcuate nucleus. Blank-SAP (Cat. #IT-21) was used as control. In animals with intact ovaries the NK3-SAP lesion resulted in hypogonadotropic hypogonadism. In contrast, the LH surge in lesioned ovariectomized rats was 3-fold higher, demonstrating that KNDy neurons are integral for the control of serum LH levels, estrous cyclicity, and may also have some control over the magnitude of the LH surge.

## Locus coeruleus noradrenergic innervation of the amygdala facilitates alerting-induced constriction of the rat tail artery.

Mohammed M, Kulasekara K, Ootsuka Y, Blessing WW. *Am J Physiol Regul Integr Comp Physiol* 310(11):R1109-19, 2016.

The researchers tested the hypothesis that release of noradrenaline within the amygdala is important for the occurrence of SCVARS (sympathetic cutaneous vasoconstrictor alerting responses). A long-shanked 5-µl glass micropipette calibrated in 100-nl steps, was filled with vehicle or Anti-DBH-SAP

(continued on page 6)

# Cerebral cholinergic lesion reduces operant responses to unpleasant thermal stimuli

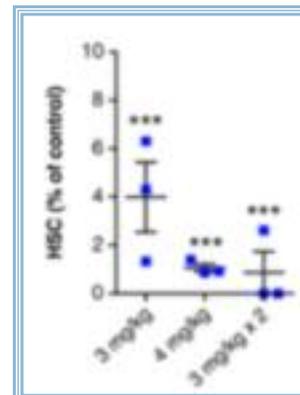
(continued from page 1)

As only hematopoietic cells express the CD45 receptor, CD45-SAP offered significant advantages with regard to toxicity compared to conventional whole body irradiation. Notably, CD45-SAP enabled quicker recovery of bone marrow cellularity, avoided damage to marrow blood vessels and other non-target marrow cells, and preserved the thymic function. Combined together, these features resulted in notably quicker recovery of B- and T-cells following CD45-SAP versus irradiation. In addition, CD45-SAP avoided neutropenia, preserving innate immunity and the ability to resist fungal infection.

To demonstrate correction of a clinically relevant disease, we employed CD45-SAP in a mouse model of sickle cell anemia and demonstrated our method achieved >90% donor cell chimerism, all mice in three groups (18/18), resulting in complete disease correction (red blood cell counts, hemoglobin levels, hematocrit levels and reticulocyte frequencies were returned to normal). Fig. 2 show hematopoietic stem cell (HSC) depletion. If these pre-clinical results can be successfully translated to the clinic, it would greatly reduce conditioning-related toxicities and expand the use of hematopoietic stem cell transplantation.

## Reference

1. Palchaudhuri R, Saez B, Hoggatt J, Schajnovitz A, Sykes DB, Tate TA, Czechowicz A, Kfoury Y, Ruchika F, Rossi DJ, Verdine GL, Mansour MK, Scadden DT. Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. *Nat Biotechnol*. 2016 Jun 6. doi: 10.1038/nbt.3584. [Epub ahead of print] PMID: 27272386.



**Fig. 2:** HSC depletion in sickle disease mice 8 days post-administration of various doses of CD45-SAP. Data represent the mean  $\pm$  SEM (n = 3 mice/dose, assayed individually).

## Recent Publications & References

(continued from page 5)

(Cat. #IT-03). Anti-DBH-SAP (5  $\mu$ g in 250 nl) or vehicle was injected into the amygdala during  $\sim$ 1 min, and the pipette was left in place for an additional 1 min. The locus coeruleus has been implicated in many aspects of emotional arousal, so that functional inhibition of the extensive locus coeruleus-derived noradrenergic innervation of centers known to be important in emotional arousal, including the amygdala, is likely to contribute to the therapeutic actions of clonidine-like agents. The locus coeruleus also has major reciprocal connections with the orexin-synthesizing

neurons in the hypothalamus, and rats with genetically lesioned orexin receptor neurons (alternatively, oen could lesion with Orexin-SAP, Cat. #IT-20) have reduced emotional arousal as reflected in reduced SCVAR responses to alerting stimuli.

### Dynamics of spinal microglia repopulation following an acute depletion.

Yao Y, Echeverry S, Shi XQ, Yang M, Yang QZ, Wang GY, Chambon J, Wu YC, Fu KY, De Koninck Y, Zhang J. *Sci Rep* 6:22839, 2016.

This study confirms that similar to microglia in the brain, spinal microglia can repopulate rapidly following elimination, which is driven essentially by a self-renewal process. To deplete microglia in spinal cords, Mac-1-SAP (Cat. #IT-06) was injected i.t. (7  $\mu$ l, 1.6  $\mu$ g/ $\mu$ l) at the level of L4-L5 in mouse. The results support the concept that

microglia repopulation, whether in the brain or in the spinal cord, is the consequence of onsite resident microglia proliferation. Newly generated microglia are fully functional and are able to respond to peripheral nerve injury and contribute to the development of neuropathic pain.

### Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin.

Palchaudhuri R, Saez B, Hoggatt J, Schajnovitz A, Sykes DB, Tate TA, Czechowicz A, Kfoury Y, Ruchika F, Rossi DJ, Verdine GL, Mansour MK, Scadden DT.

*Nat Biotechnol* 2016.

see cover article

Did we miss you?  
If so, please send a PDF  
of your publication to  
admin@atsbio.com so we  
can feature your research in  
our next issue!

# Talking about Targeting

## Streptavidin-ZAP Applications

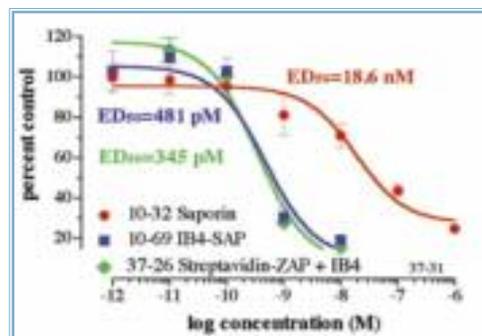
**Q:** I've been looking at your secondary conjugates and want to see if my targeting agent is specific to certain cells. Which secondary conjugate should I use?

**A:** It depends on two factors: 1) the type of assay you want to use, and 2) the kind of targeting agent you want to use.

For *in vitro* assays, in particular, internalization assays, you can use any of the ZAP Internalization Kits (Z-Kits that include all the materials necessary to test your targeting agent). For the Secondary Antibody Z-Kits, you use your primary antibody and select the appropriate secondary antibody species (e.g. for a human antibody, use Hum-ZAP (Cat. #KIT-22-Z), Fab-ZAP human (Cat. #KIT-51-Z), FabFC-Human (Cat. #KIT-65-Z), Hug-M-ZAP (Cat. #KIT-43-Z), or Fab-ZAP Hug-M (Cat. #KIT-78-Z), depending on the isotype of your primary antibody ([See page 8 Promos](#)). Or you can biotinylate your antibody and use Streptavidin-ZAP (Cat. #KIT-27-Z).

For *in vivo* applications, it depends on the kind of targeting agent you want to use. Regardless of whether you use an antibody, peptide or ligand, you will need to biotinylate the material first. ATS offers a biotinylation service that is efficient and economical ([See page 8 Promos](#)).

If you are using a biotinylated peptide, you will use a kit that includes the appropriate control -- Blank-Streptavidin-SAP. Order Cat. #KIT-27-B (25 mcg, 100 mcg, 250 mcg, or 1 mg).



KNRK cells are plated at 2500 cells/well and incubated overnight. Streptavidin-ZAP is premixed with Biotinylated-IB4 in equimolar concentrations. Saporin, IB4-SAP, and the Streptavidin-ZAP+ Biotinylated IB4 mixture are then added in 10- ml volumes and the plates are incubated 72 hours. PMS/MTS developing reagent is added and the plates are incubated 15-30 minutes, then read at 490 nm.

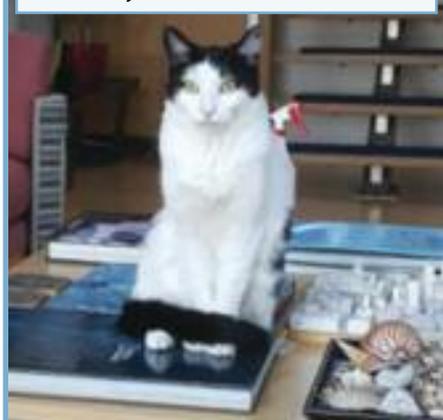
If you are using a biotinylated antibody, you will use the Streptavidin-ZAP kit that includes the appropriate antibody species control:  
*BIGG-SAP Human, BIGG-SAP Mouse  
 BIGG-SAP Rabbit, BIGG-SAP Rat*

Select the kit that matches the species of your biotinylated primary antibody. Each kit is available in 25, 100, 250 or 1000 mcg sizes.

*KIT-27-Ahu - human antibody  
 KIT-27-Amu - mouse antibody  
 KIT-27-Arb - rabbit antibody  
 KIT-27-Art - rat antibody*

**Have questions?  
 Email us at [admin@ATSbio.com](mailto:admin@ATSbio.com)**

**Kermit, the Coffee Table Cat**



## Where to See Us:

### Pain Summit

Chicago, October 19-20

### Society for Neuroscience

San Diego, November 12-16

Tollfree Phone: (877) 889-2288  
Phone: (858) 642-1988

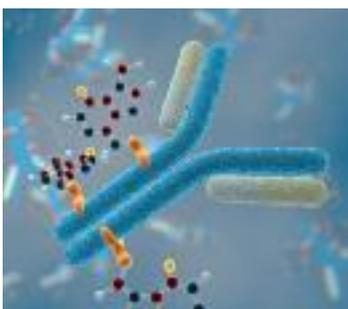
[www.ATSBio.com](http://www.ATSBio.com)

Monday-Friday 9am-5pm (PT).  
Order online, via telephone or  
fax, or with your local distributor.



10451 Roselle Street #300  
San Diego, CA 92121

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



A targeting agent is conjugated to a payload. Here the conjugate is an antibody linked to a small molecule.



The targeted conjugate binds only to the cells that are specific to the antibody causing the receptor to internalize.



The internalized conjugate is digested by enzymes releasing the payload into the cytosol. The payload is then free to alter the cell.

## Daily Promotions on Targeting Tools

• **Check our website daily** •

*Promotion offers available when you order online*



Brian Russell

### Z-Kit Summer – ZAP Internalization Kit offer

Z-kits include everything you need to perform cell-based internalization assays with your antibodies or biotin-labeled material. Buy any ZAP Internalization Z4 Kit (400 tests) and get an original Z-Kit (100 tests) for free,

or

Buy any ZAP Internalization Z10 Kit (1000 tests) and get a Z4 Kit for free. Mix or Match, your kit can be the same or choose another from our ZAP Internalization Kit catalog. Enter coupon code **ZKITSUM** at checkout. Offer expires July 31, 2016.

### Biotinylation Bonanza – Streptavidin-ZAP offer

Buy a 100-mcg size of Streptavidin-ZAP (IT-27-100) and get an instant upgrade to an Internalization Kit (KIT-27-Z100). Z-kits include everything you need to perform internalization assays with your biotinylated targeting agent. Enter coupon Code **BIOTIN-Z** at checkout.

**And** a Special Biotinylation Offer: Get 50% off a biotinylation service when you order a Streptavidin-ZAP Internalization kit. Enter coupon code **BIOTIN-Z50** at checkout. Offers expire July 31, 2016.



Leonardo Archeta



Chelsea Friedman

### Melanopsin Products – you will see double

Order any Melanopsin product and you will receive 2X the size you order. Enter coupon Code **DBLOPSN** at checkout. Offer expires July 31, 2016.

**Go to [ATSBio.com](http://ATSBio.com) to stretch your budget.**

It's easy. Click on **My Account** and register now.

Use promo code **WEB2016** and get 10% off your first online order.