Navigating the Turbulent Waters of the Pandemic

2020 ushered in a new way of life for all of us. The first COVID case was confirmed by the Center for Disease Control (CDC) on January 17. In California, where Advanced Targeting Systems is located, on March 1st, masks were ‘strongly recommended’ in indoor settings. On March 11, the World Health Organization (WHO) gave pandemic status to COVID-19. By March 19th, a statewide ‘Stay-at-Home’ order was given.

Around the world, countries and communities were thrust into turmoil trying to get ahead of the virus in a number of different ways. Finding enough protective gear for medical workers dealing with the onslaught of new cases, finding beds and respiratory equipment to take care of hospitalized patients, developing diagnostic tests and vaccines, and enforcing strict guidelines to protect everyone.

For ATS, this meant setting up schedules for employees to work from home, providing masks and hand sanitizer when laboratory work had to be done, and keeping our products and services available to the researchers still in laboratories working on solutions to end the pandemic. Since ATS was working and providing services for development of diagnostics and treatments for COVID-19, we were able to get our employees vaccinated fairly quickly. This was not the case for many of you, and the isolation this caused was devastating.

ATS put many protective measures in place to ensure that there was a clean work environment for our team members. Hand sanitizers and masks were provided for employees and their extended families. We even offered free masks for anyone placing an order.

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Life in the Pandemic

It was fortunate for ATS that we had just relocated to Carlsbad a month before the pandemic was making an impact in southern California. The new space was set up with an open office design where nobody’s desk was closer than six feet to the nearest neighbor. My primary concern was making sure everyone had Personal Protective Equipment (PPE) to give them a level of comfort that they could come in to work and not be exposed to COVID. In the early days there was not much available on the market and what was there was being ravaged by frightened people or, unfortunately, people who wanted to make a profit by reselling at twice the original price. Since I majored in Theatre in college and had good experience in costume making, I thought I’d just investigate the best and safest mask designs and make those for our team and their significant others. It kept me busy outside of business hours and I was also able to give masks to some of the healthcare workers who were also experiencing difficulties in getting the necessary PPE.

We quickly put Zoom meetings into action and were able to hold our weekly meetings and meet with scientists from other institutions without leaving our desks. It allowed us the opportunity to get creative with our presentations so that everyone could see and hear quite well. Whether for business or personal reasons, Zoom allowed us to celebrate occasions with others and have multiple attendees sharing with each other—seeing each other, laughing and enjoying a new kind of social life.

Outdoor activities became the safest way to exercise and socialize. I enjoyed playing golf twice a week, even though we were required to wear a mask and benches and golf ball-washing stations were removed. The upside was that they also removed the rakes from the sand traps and the COVID rules stated you must remove your ball from the sand and place it nearby on the grass. My scores were better than ever!

Looking forward, we’ve learned some valuable lessons. Wearing a mask, washing your hands frequently and keeping a 6-foot distance from others actually helps you become generally more healthy – fewer colds and other common sicknesses. I’m looking forward to healthy days ahead.

With the pandemic came the slowdown of social activities, which gave me more time to read, workout, cook and try new hobbies like making jewelry. It was definitely a time of exploration and appreciation. The pandemic was a good reminder to not take your health and people in your life for granted, and to recognize how fortunate we are to live in a country that can take care of us – even though it was hard to find toilet paper 😞. I am glad that I was able to stay connected to family and friends who live across the world, since travelling was not an option.

The pandemic started with extra company when my two college-age children were suddenly home for a few extra months. As the days stretched on, I started reading more than ever. And then we did what many others did and got a new puppy! When we weren’t busy taking him for walks, my husband Mason and I also worked on renovating our house.
At the start of 2020, home-life was trying to raise a 3 and 5 year old along with caring for my wife’s parents who live with us. By late November, our household was trying to manage the domino effect of one of us contracting the virus and ultimately spreading it to the rest household. Looking back at the challenges, we’re seeing how the pandemic helped tip-the-scales regarding certain decisions…and all for the better. My wife and I solidified our decision to homeschool both our kids, where everyone, including my wife, seem to be thriving. Mom and Dad consider themselves lucky and try never to miss a moment of enjoying their grandchildren with tighter hugs, and ATS reminded me that my ‘workplace’ was more than the place that I work, but a place of support and friendship.

Many little life lessons were learned and reaffirmed during the 2020 Pandemic:

• Smile, even when your mask is covering your face, and no one can see.
• Patience. Standing in long masked-up lines for cleaning products, bathroom tissue and vaccine shots.
• Understanding, acceptance without shame that CDC guidelines for many were blurry. Let me do what I need to do to feel safe and you do what you need to do to be comfortable.
• Acceptance that this Pandemic lifestyle may have many on and off sequences regarding masking and staying away from crowds.
• Trusting in others. The few people in my sphere I believe all behaved in a fiduciary manner with regard to my health including written and unwritten agreed upon behaviors and covenants.
• Information. Too much information wasn’t the best goal, but reliable sources were.
• Yes, more than a few daily simple life choices changed. Many other life tools were redefined and reaffirmed.

The negatives of the pandemic have been countless. My personal experience was that I struggled with the lack of interaction with friends and family during peak mandates. There have been some bittersweet aspects of the pandemic, like spending more time doing things like gardening and backpacking. Being an essential worker, I still had to come into the lab, but fortunately there was none of the usual traffic on my commute. I even picked up a few new hobbies; at one point I had a sourdough starter and beer fermenting in my kitchen – felt like I was starting a second lab at home. At the end of the day, I feel lucky to have had a dog throughout the pandemic.

Objective: The authors use zebrafish to conduct a deeper analysis of the potential adverse effects of donepezil (DPZ) on the short-term memory and behaviors of normal zebrafish by performing multiple behavioral and biochemical assays.

Summary: Chronic waterborne exposure to DPZ can severely induce adverse effects on normal zebrafish in a dose-dependent manner.


Extracellular levels of the sleep homeostasis mediator, adenosine, are regulated by glutamatergic neurons during wakefulness and sleep. Sun MJ et al. Purinergic Signal 16(4):475-476, 2020.

Objective: To clarify the role of septal input in the development of long-term potentiation (LTP) in this synapse.

Summary: Elimination of septal cholinergic neurons by 192-IgG-SAP abolished LTP development in both experimental series. This suppression of LTP in animals with a cholinergic deficit was not due to loss of hippocampal neurons.

Dose: Rats received intraseptal injections of 192-IgG-SAP (1.5 μg).


Summary: A plethora of studies conducted in rodents demonstrated that selective lesions of BF cholinergic neurons and their cortical inputs produced by the immunotoxin 192-IgG-SAP impair performance in various tasks of attention.

Objective: To determine if Object Recognition Memory (ORM) can be restored.

Summary: Drug-induced effects have been used to demonstrate the construct validity of operant attention tasks, as well as to assess the pharmacological systems that underlie cognitive processes, such as attention, short-term memory and reaction time, either by interrupting or enhancing performance.


Objective: To determine if Object Recognition Memory (ORM) can be restored.

Summary: Review of lesioning designed to specifically target cells that express acetylcholine as a transmitter.

### 2020 Scientific References

Postnatal development. Patrone L et al.


**Objective:** To confirm hypothesis that dysfunctional descending noradrenergic and serotonergic pain control circuits may contribute to the loss of diffuse noxious inhibitory control (DNIC), a critical endogenous pain control mechanism, weeks to months after traumatic brain injury (TBI).

**Summary:** Results suggest that TBI causes maladaptation of descending nociceptive signaling mechanisms and changes in the function of both adrenergic and serotonergic circuits. Such changes could predispose those with TBI to chronic pain.

**Dose:** Anti-DBH-SAP (5 μg/5 μl) was injected in the left ventricle. Lesion of the LC resulted in failure of DNIC, an effect that mimics what is observed behaviorally after chronic TBI.

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**Summary:** Ablation of norepinephrine containing projections to the arcuate (via Anti-DBH-SAP injections) alters AgRP and neuropeptide (NPY) concentrations, leading to impairments in hypoglycemic (glucoprivic) or ghrelin-induced feeding.

**Dose:** Either 5μL of 40 ng/μl Anti-DBH-SAP or Mouse IgG-SAP (control) was applied three times directly to the exposed apical/anterior surface of the heart.

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**Integration of peripheral and central systems in control of ingestive and reproductive behavior.** Schneider J Oxford Research Encyclopedia of Neuroscience , 2020. Oxford University Press

**Objective:** To determine if the ablation of C1 neurons mitigates high blood pressure induced by high-salt intake.

**Summary:** Data show that hypertension induced by high-salt intake is dependent on C1 neurons.

**Dose:** Bilateral injections of 2.4 ng/100 nl of Anti-DBH-SAP. The total number of TH+ neurons in the AS region was reduced by 37 ± 13% in the anti-DBH-SAP group when compared to control.

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**Targeting Trends 2020 Review - 5**

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**Objective:** To discover the role of brainstem catecholaminergic (CA) neurons in the hypoxic ventilatory response (HVR).

**Summary:** Brainstem CA neurons modulate the HVR during the postnatal phase, and possibly thermoregulation during hypoxia.

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**Summary:** Data show that catecholaminergic projections from glucose-sensitive cells in the ventrolateral medulla are necessary for all responses to glucoprivation, including increases in epinephrine secretion, glucocorticoid secretion, sex behavior, and food intake.

**See:** Ritter S Physiol Behav 208:112568, 2019.

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**2020 Scientific References**

**IT-06, IT-33 Mac-1-SAP**


**Objective:** To understand the mechanism responsible for this difference in magnitude of phrenic long-term facilitation (pLTF)

**Dose:** For saporin-mediated depletion of macrophages, DO-surgery-treated mice received an intraventricular (iv) injection of either Mac-1-SAP or Rat IgG-SAP (20µg) once every 3 days.


**See:** Gulino R Neural Plast 158:1792-1801, 2017.

**IT-11 SSP-SAP**


**Objective:** To describe how exercise is neuroprotective for motoneurons, accelerating axon regeneration following axotomy and attenuating dendritic atrophy following the death of neighboring motoneurons.


**Dose:** Targeted toxin lesions across three sites of the paratrigeminal nucleus (200nl per injection site).

**See:** Gulino R Neural Plast 2016:2769735. 2016.

**IT-14 CTB-SAP**


**Summary:** pLTF in 7d CTB-SAP treated rats is elicited primarily through TrkB and PI3K/Akt-dependent mechanisms, whereas BDNF and MEK/ERK-dependent mechanisms induce pLTF in 28d CTB-SAP treated rats.

**Dose:** Rats received bilateral intrapleural injections of CTB-SAP; 25 µg dissolved in PBS.

**IT-23 Anti-SERT-SAP**


**Objective:** To investigate the role of amygdala-projecting 5-HT neurons in the DR in innate anxiety and conditioned fear behaviors.

**Dose:** Each rat received two bilateral microinjections per site (100 nL each, 1 µM in artificial cerebrospinal fluid) of either Anti-SERT-SAP or the control Mouse IgG-SAP.

**Summary:** Targeting gastrin-releasing peptide receptor (GRPR) and natriuretic peptide receptor A (NPRA) contribute to chronic itch in a murine model of allergic contact dermatitis. Liu X et al. J Invest Dermatol 140(9):1856-1866.e7, 2020.

**Objective:** The authors investigated the peripheral and spinal mechanisms responsible for prolonged itch in a mouse model of allergic contact dermatitis (ACD) induced by squaric acid dibutylester (SADBE).

**Summary:** Targeting gastrin-releasing peptide receptor (GRPR) and natriuretic peptide receptor A (NPRA) may provide effective treatments for ACD associated chronic pruritus.

**Dose:** A single dose of Bombesin-SAP (400 ng) and Blank-SAP (400 ng) or two doses of Nppb-SAP (BNP-SAP; 650 ng) and Blank-SAP (650 ng) were administered via intrathecal injection.

**IT-40 Bombesin-SAP**


**Summary:** Tongue muscle weakness results in dysarthria and dysphagia leading to recurrent aspiration, choking, and aggravation of respiratory disease.

**See:** Lind LA et al. Neuroscience 390:303-316, 2018.

**Objective:** To understand the role of paratrigeminal neurokinin 1 receptor neurons in the regulation of cough, breathing and airway defensive responses.

**Dose:** Targeted toxin lesions across three sites of the paratrigeminal nucleus (200nl per injection site).

**Summary:** In a mouse model of distraction osteogenesis (DO), there was significant increase in macrophages in the regeneration area. This suggests that targeting inflammatory macrophages may help to improve clinical bone repair.


**Objective:** This study aimed to assess the involvement of paratrigeminal neurokinin 1 receptor neurons in the regulation of cough, breathing and airway defensive responses.


**Summary:** The authors refer to a review by Gulino describing two rodent models of motoneuron degeneration were induced by neurotoxics including volkensin and cholera toxin-B saporin, which are able to destroy motoneurons through retrograde transportation.

**See:** Gulino R Neural Plast 2016:2769735, 2016.

**Objective:** To describe how exercise is neuroprotective for motoneurons, accelerating axon regeneration following axotomy and attenuating dendritic atrophy following the death of neighboring motoneurons.

**Summary:** Exercise offers a simple, low cost and effective non-pharmacological approach to improve clinical bone repair.

2020 Scientific References


Objective: To determine the role of GRP in sensory neurons.

Summary: GRP is a neuropeptide in sensory neurons for nonhistaminergic itch, and GRP sensory neurons are dedicated to itch transmission.

Dose: Bombesin-SAP (200 ng/5 μL, i.t.) was injected 2 weeks prior to optical stimulation.


Summary: The discovery of descending neural circuitry to drive the itch-scratching cycle may provide potential therapeutic targets in the central nervous system for the management of chronic itch.

Dose: To ablate the spinal GRPR+ neurons, mice were intrathecally injected with Bombesin-SAP or Blank-SAP (400 ng/5 mL).


IT-42 Anti-ChAT-SAP

Summary: Basal extracellular levels of dopamine within the nucleus accumbens are not sustained by muscarinic acetylcholine, whereas accumbal Cholinergic interneurons-ACh are involved in mediating ethanol-induced dopamine release.

Dose: Anti-ChAT-SAP or Rabbit IgG-SAP were infused at a flow rate of 0.05 μl/min for 10 min giving a total volume of 0.5 μl.

IT-47 Leptin-SAP

Objective: This study tested whether loss of hindbrain leptin receptor signaling changed sensitivity to forebrain leptin.

Summary: Loss of VMH (ventromedial nucleus of hippocampus) leptin receptor-expressing cells prevents weight loss. The integrated response between the hindbrain and forebrain is heavily dependent upon leptin activity in the VMH.

Dose: To test forebrain leptin sensitivity Leptin-SAP and Blank-SAP rats received third ventricle injections of 0, 0.05, 0.1, 0.25 or 0.5 μg leptin.


IT-69 Nppb-SAP

Summary: Mice treated with Nppb-SAP ablated 70% of the BNP receptor-positive neurons in the spinal cord.


Objective: Review of recent findings used to examine the role of B-type natriuretic peptide (BNP) in itch transduction and the modulation of other pururitic proteins.

Summary: The authors review the functions of the kappa opioid receptor (KOR) and its endogenous agonists dynorphins in modulating itch and pain. Nppb-SAP ablation of neurons expressing the Natriuretic olypeptide B receptor greatly reduced itch responses evoked by histamine or by intrathecal administration of Nppb, suggesting these neurons transmit itch signals from Nppb primary afferents.


Reviews

A collection of 20 articles that depict a broad representation of the most impactful advances in Alzheimer’s disease (AD) comprehension and therapeutic openings.


A review of studies where saporin-based conjugates were used to analyze cell mechanisms of sleep, general anesthesia, epilepsy, pain, and development of Parkinson’s and Alzheimer’s diseases.

Objective: To determine if targeting Siglec-8 with mouse IgG1 antibodies, rather than with rat IgG antibodies, in mice transgenic for Siglec-8, will prove to be a more effective strategy for eliminating mouse eosinophils in vivo.

Summary: This study is the first to describe a novel mouse strain of Siglec-8+F− eosinophils—a useful tool for studying human Siglec biology in preclinical models.

Dose: Siglec-8+F− mouse eosinophils were pretreated with 10 μg/mL saporin-conjugated isotype controls (mouse IgG1 or rat IgG2), anti-Siglec-8 (2C4) or anti-Siglec-F (9C7) antibodies for 24 h.


Objective: The authors describe how nanometer-sized fluorescent semiconductors called quantum dots (QD) can be used to label neuronal proteins in a single QD imaging format.

Dose: Secondary antibody-conjugated QDs target membrane proteins pre-treated with SERT Mouse Monoclonal.


Dose: bilateral injections of urotensin II-saporin (UII-SAP; 0.07 μg/μL per site – unless stated otherwise; generous gift from Advanced Targeting Systems)

Saporin


Summary: This study reviews the characterization of fever and sickness behavior regulated by cytokines during infection.

Dose: IL-1β-saporin or unconjugated Saporin as control (icv or ip, 1.75 μg) eliminated IL-1R1-expressing cells in the hippocampus and indicated these neurons mediate the function of peripheral IL-1β induced hypophagia.


Summary: Extracellular vesicles (exosomes, EVs) with encapsulation of biofunctional molecules (e.g., enzymes and genes) are highly expected to be next-generation therapeutic carriers because of their pharmaceutical advantages. Saporin-artificially encapsulated EVs with modification of the (sC18)2 peptides showed glycosaminoglycan-dependent cell-killing activity. Our experimental techniques and findings are considered to contribute to the development for EV-based intracellular delivery system via macropinocytosis.

IT-17 Rat IgG-SAP


See IT-06 Mac-1-SAP

IT-18 Mouse IgG-SAP


See IT-03 Anti-DBH-SAP


See IT-23 Anti-SERT-SAP

IT-35 Rabbit IgG-SAP


See IT-42 Anti-ChAT-SAP
ZAP Secondary Conjugates

ZAP conjugates are non-targeted saporin conjugates that "piggyback" on to your primary targeting agent

**IT-27 Streptavidin-ZAP**


*Summary:* Refers to chimeric murine-human mAb cetuximab bound to Streptavidin-ZAP.


**ALPPL2**


**Objective:** To evaluate therapeutic potential of ALPPL2 targeting.

**Summary:** Exquisite tissue specificity and broad tumor type coverage suggest that ALPPL2 could be an excellent cell surface target for therapeutic development against mesothelioma.

**Dose:** Biotinylated M25 IgG1 and Streptavidin-ZAP were mixed at a molar ratio of 1:1.

**IT-04 Mab-ZAP**


*Summary:* The increased and sustained expression of TCblR in proliferating cells has been used to target toxins preferentially to cancer cells and can be potentially used for targeted delivery of other anti-cancer drugs. In 2010 the authors published a paper which evaluated the potential of using immunotoxins to eliminate cancer cells expressing TCblR the authors performed a series of in vitro experiments using their monoclonal antibody plus Mab-ZAP in varying concentrations. The results indicated that this is a viable therapeutic model that causes minimal peripheral damage.


*Summary:* The authors review the most current mAb-based therapies against EphA2-expressing cancers currently in pre-clinical and/or clinical stages. They reference Sakamoto et al. who performed in vitro testing of two different EphA2 mAbs mixed with Mab-ZAP to discover their therapeutic potential against melanoma.

**ZAP Secondary Conjugates**

ZAP conjugates are non-targeted saporin conjugates that "piggyback" on to your primary targeting agent

**AB-N27AP Anti-Angiotensin II receptor**


**Western blot**


**Immunohistochemistry** (1:800)


**Immunohistochemistry**

**AB-N38 Anti-Melanopsin**


**Immunostaining:** 1:10000


**Retinal immunohistochemistry** (1:5000)

**AB-T044 Anti-Conj Trans-4-Hydroxy-L-Proline**


**Western blot**


**Immunoprecipitation**


**Western blot (1:500)**

**AB-T125 Anti-Conjugated NO-L-Cysteine**


**Immunofluorescence**

**BT-17AP Biotin-labeled Anti-Saporin**

Top 20 Targeting Tools of 2020

Top 5 Targeted Toxins

#1. CTB-SAP (Cat. #IT-14)  
targets cells expressing GM1 receptor
#2. Anti-DBH-SAP (Cat. #IT-03)  
targets cells expressing rat dopamine beta-hydroxylase
#3. IB4-SAP (Cat. #IT-10)  
targets cells expressing alpha-D-galactopyranoside residues
#4. mu p75-SAP (Cat. #IT-16)  
targets cells expressing mouse p75NTR
#5. ME20.4-SAP (Cat. #IT-15)  
targets cells expressing multiple mammalian species of p75NTR

Top 5 ZAP Products

#1. Streptavidin-ZAP (Cat. #IT-27)  
Uses your biotinylated material
#2. Fab-ZAP human (Cat. #IT-51)  
Uses your primary human monoclonal antibody
#3. Fab-ZAP mouse (Cat. #IT-48)  
Uses your primary mouse monoclonal antibody
#4. Fab-ZAP rat (Cat. #IT-55)  
Uses your primary rat monoclonal IgG antibody
#5. FabFc-ZAP human (Cat. #IT-65)  
Uses your primary human monoclonal IgG antibody

Top 5 in Number of Publications

#1. 192-IgG-SAP (Cat. #IT-01)  
targets cells expressing rat p75NTR
#2. Anti-DBH-SAP (Cat. #IT-03)  
targets cells expressing rat dopamine beta-hydroxylase
#3. CTB-SAP (Cat. #IT-14)  
targets cells expressing GM1 receptor
#4. SSP-SAP (Cat. #IT-11)  
targets cells expressing substance P receptor (NK-1)
#5. Bombesin-SAP (Cat. #IT-40)  
targets cells expressing express gastrin releasing peptide (GRP) receptor

Top 5 Antibodies

#1. Melanopsin Rabbit Polyclonal, affinity-purified (Cat. #AB-N39)
#2. Anti-Conjugated Trans-4-Hydroxy-L-Proline (Cat. #AB-T044)
#3. Angiotensin II receptor (AT-2R) Rabbit Polyclonal, affinity-purified (Cat. #AB-N28AP)
#4. NGFr (mu p75) Rabbit Polyclonal, affinity-purified (Cat. #AB-N01AP)
#5. Angiotensin II receptor (AT-1R) Rabbit Polyclonal, affinity-purified (Cat. #AB-N27AP)