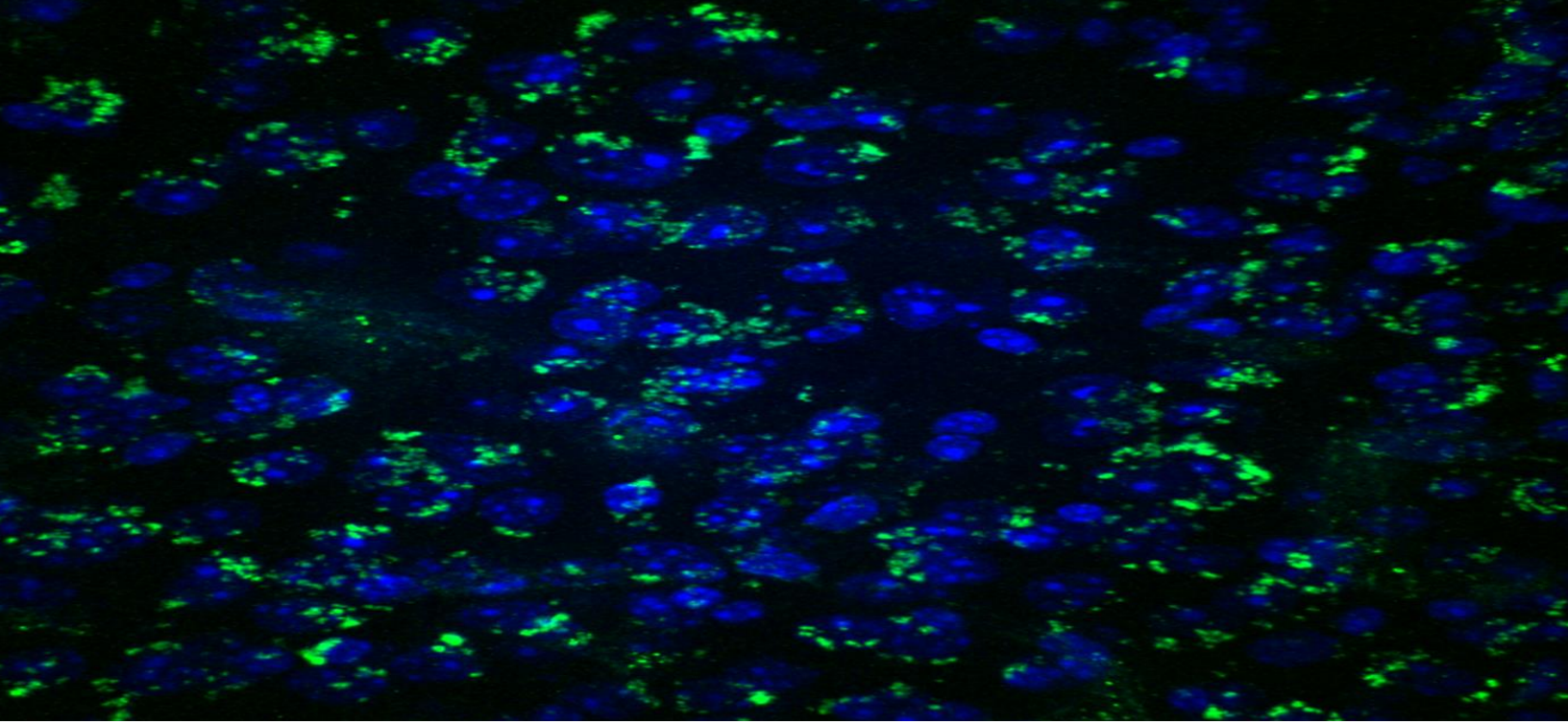

Inflammasome ASC

A Promising Therapeutic Target

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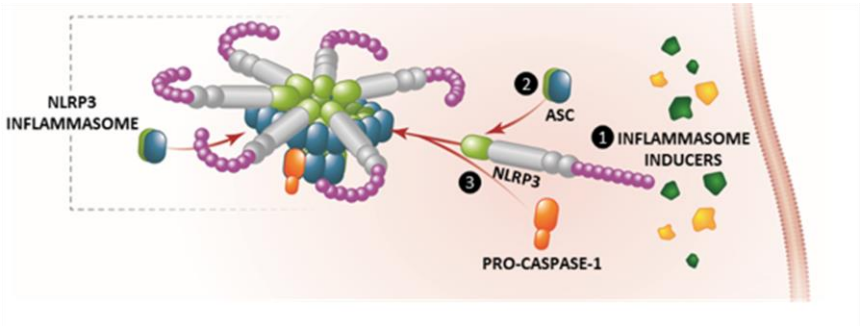
Background

Inflammasomes are intracellular multiprotein complexes of the innate immune system that initiate inflammation in response to either exogenous pathogens and viruses or endogenous danger signals. The role of inflammation is to provide protection by eliminating pathogens and promoting tissue repair and recovery. A normal inflammatory response is temporary and resolves once the threat has passed. In some circumstances, the inflammatory response is prolonged. Shifts in the inflammatory response from short- to long-term can lead to major alterations in tissues, organs, and cellular physiology, and are associated with numerous and diverse chronic inflammatory diseases. Chronic inflammatory diseases have been recognized as the most significant cause of death in the world today, with more than 50% of all deaths being attributable to inflammation-related diseases such as ischemic heart disease, stroke, cancer, diabetes mellitus, chronic kidney disease, non-alcoholic fatty liver disease, and autoimmune and neurodegenerative conditions.[1]

Inflammasome complexes are comprised of three proteins: a sensor protein, adaptor ASC, and effector pro-caspase-1. There are at least 12 sensor proteins that each respond to different pathogens and danger signals.[2] Inflammasomes are named after their associated sensor protein. Activated sensor proteins trigger assembly of ASC proteins, which in turn recruit pro-caspase-1 to form an inflammasome as depicted below.

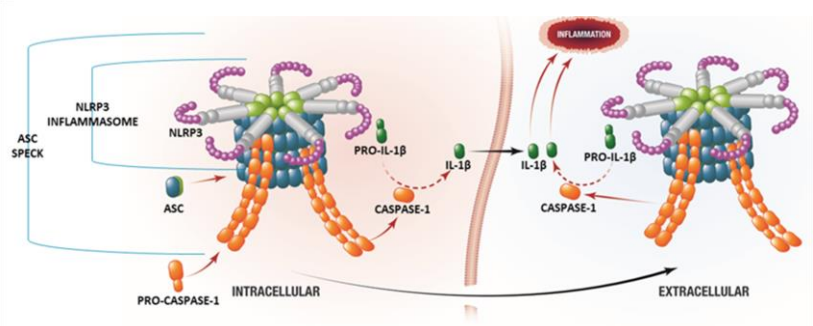
Inflammasome Formation



Inflammasomes serve as a docking platform for additional ASC proteins which polymerize to form a large filamentous structure known as an ASC speck. ASC specks provide a scaffold for pro-caspase-1, promoting its activation and subsequent maturation of cytokines IL-1β and IL-18 thereby initiating the inflammatory process.

Caspase-1 also activates Gasdermin D, triggering a form of cell death known as pyroptosis. Following pyroptosis, cellular content, including active cytokines and ASC specks, is released into the extracellular space as depicted below.

ASC Speck Formation & Pyroptotic Release



Extracellular ASC specks remain active, continuing to release IL-1β and IL-18. They can also be engulfed by surrounding cells and act as danger signals to activate and trigger an inflammatory cascade in these cells.

Inflammasome activation and the associated secretion of proinflammatory cytokines IL-1β and IL-18 are required to respond to and initiate adaptive responses against pathogens, but inflammasome hyperactivation and/or prolonged activity can cause damaging inflammation contributing to a broad range of inflammatory conditions and diseases.

Inflammasome ASC as a Therapeutic Target

ASC, in monomeric form or as ASC specks, plays a critical role in initiation, amplification, and perpetuation of the inflammatory response, and it acts as a bridge between innate and adaptive immunity.[2]

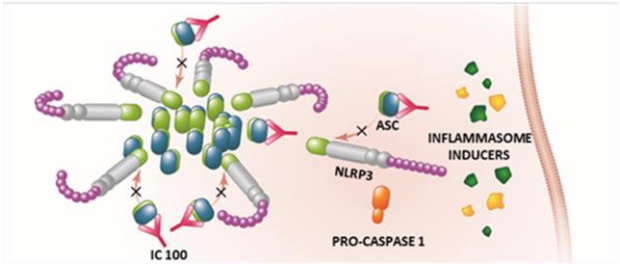
ASC is central to formation and activation of different inflammasome complexes, associated with up to 12 sensor molecules[2] (NLRP1, NLRP2, NLRP3, NLRP6, NLRP7, NLRC4, NLRC5, NAIP2, NAIP5, NAIP6, Pyrin,and AIM2). ASC inhibition across multiple inflammasome complexes is expected to better control inflammation in disorders and diseases associated with activation of more than one type of inflammasome complex, as exemplified below.

Multiple Inflammasomes Trigger Many Diseases/Conditions

Disease	Inflammasomes Activated
Obesity	AIM2, NLRP3
Insulin Resistance	AIM2, NLRP1, NLRP3, NLRC4, NLRP6
Diabetic Nephropathy	AIM2, NLRP3
Atherosclerosis	NLRP1, NLRP3, NLRC4
Alzheimer's Disease	AIM2, NLRP1, NLRP3
Multiple Sclerosis	AIM2, NLRP1, NLRP2, NLRP3, NLRC4

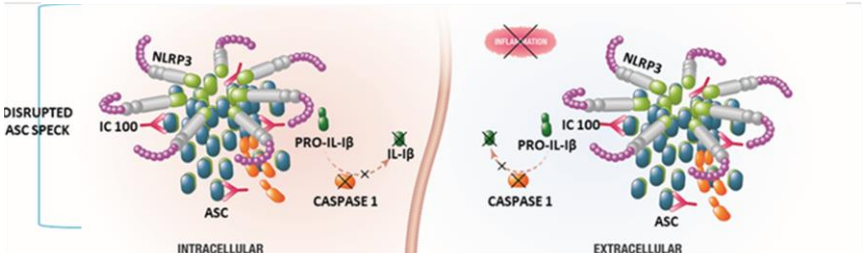
ASC inhibition will block inflammasome formation to stop initiation of the inflammatory cascade as depicted below with IC 100.

Blocked Inflammasome Formation



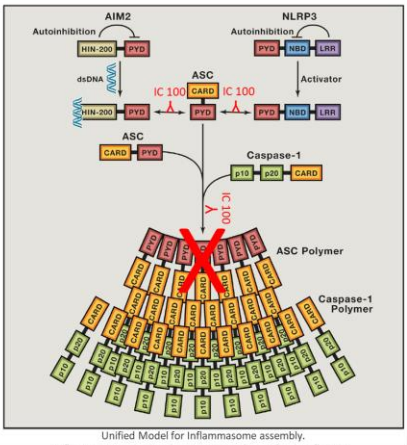
ASC inhibition will disrupt the structure and function of ASC specks, intracellularly and extracellularly, as depicted below with IC 100, blocking perpetuation of systemic inflammation.

Disrupted ASC Speck Architecture



Inflammasome ASC Inhibitor IC 100

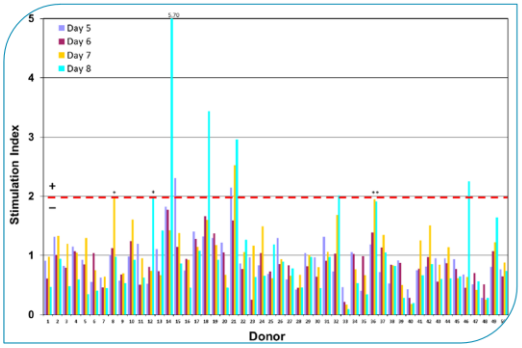
Inflammasome ASC Inhibitor IC 100 was developed by authors of this White Paper from the University of Miami Miller School of Medicine to address heightened and prolonged inflammasome activation that perpetuates damaging inflammation underlying numerous CNS and other inflammatory conditions. IC 100 is a humanized monoclonal IgG4 antibody that binds to the PYD domain of adaptor ASC to block PYD-PYD molecular interactions across multiple types of inflammasome complexes. As seen in the image below, by blocking PYD-PYD interactions, IC 100 prevents ASC binding to sensor proteins to block inflammasome formation, and it disrupts intracellular and extracellular formation of ASC specks to stop initiation and perpetuation of inflammation.



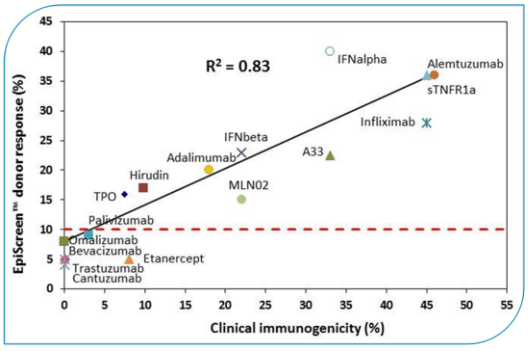
Unified Model for Inflammasome assembly.
Inflammasome: Putting the Pieces Together. Ruland, Cell, 2014

IC 100 was designed to be devoid of T-cell epitopes to reduce the risk of immunogenicity. Reduced immunogenicity was demonstrated in epigenetic screening performed by Antitope, Ltd. In this assessment, T-lymphocytes obtained from 50 healthy volunteers were treated with IC 100, and T-cell blast response was assessed by H3thymidine uptake and IL-2 production. These values were used to formulate an immunogenicity index. The graphs below show the healthy donor T-cell proliferation response and the immunogenicity index for IC 100 in comparison to common mAbs. At 9%, the immunogenicity index for IC 100 was lower than that of most of the mAbs evaluated.

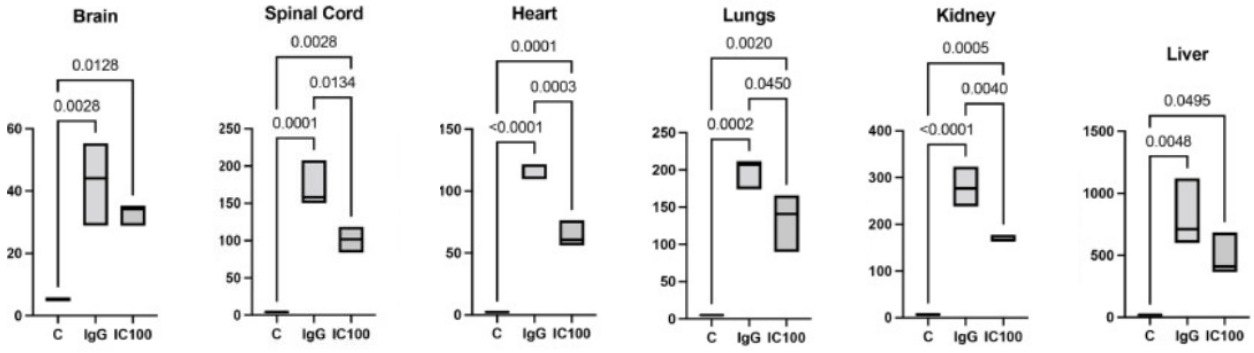
T-cell Proliferation Response



IC 100 Immunogenicity Index



IC 100 has broad tissue distribution as demonstrated by fluorescent labeling and *in vivo* imaging in B6 albino mice.[3] IC 100 crossed the blood/brain barrier, and as depicted below, demonstrated significantly higher distribution than controls in brain, spinal cord, heart lungs, kidneys and liver, suggesting potential as a therapeutic option in a broad range of CNS and non-CNS indications.

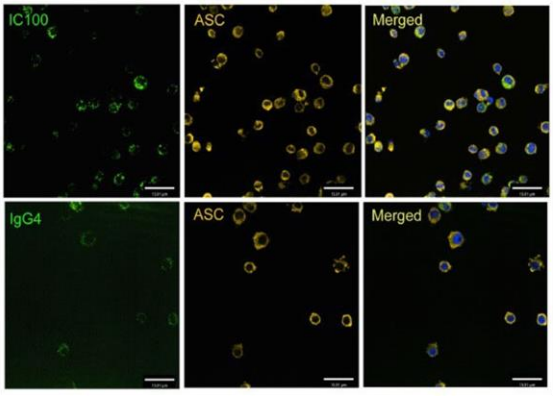


Units: Average radiant efficiency (photons/s/cm²/sr/µW/cm²); MEAN ± SEM

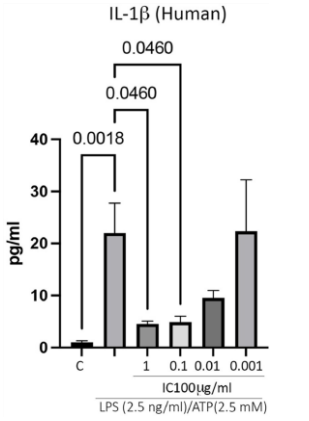
IC 100 Mechanism of Action[3]

IC 100 binds to intracellular ASC and inhibits IL-1β release as depicted below. Binding to ASC was demonstrated by confocal imaging of immortalized bone marrow-derived macrophages that were treated with 5 µg/mL of FITC-conjugated IC 100 or an IgG4κ isotype control for 1 hour and then double-labeled with anti-ASC and Alexa-flour 594-conjugated secondary antibodies. Inhibition of IL-1β release was demonstrated by adding IC 100 at different concentrations to whole human blood stimulated with 2.5 mg/mL of LPS for 3.5 hours followed by 2.5 mM ATP for 30 minutes.

IC 100 Associated with Intracellular ASC, IgG4 Did Not

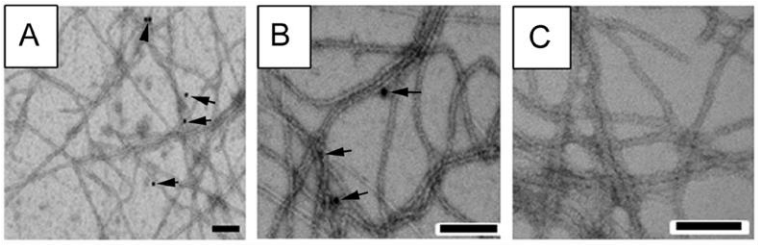


IC 100 Inhibited IL-1β In Dose-dependent Manner



IC 100 binds to ASC^{PYD} filaments as demonstrated by immunogold labeling depicted in the image below.

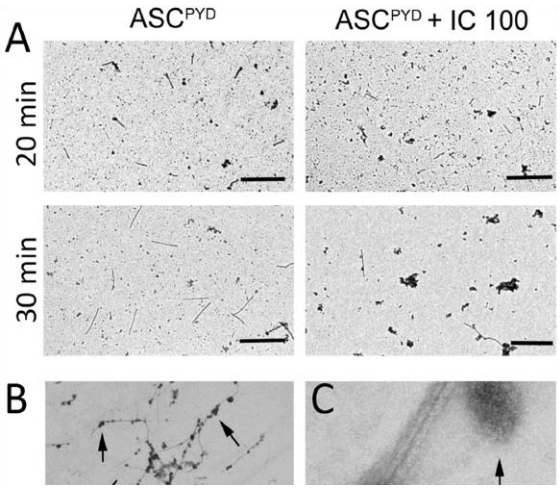
IC 100 Binds to ASC^{PYD} Filaments



- Immunogold-conjugated IC 100 labeled ASC filaments primarily at the ends of the filament and at various locations along the length of the filament (Figures A and B, arrows)
- The control unlabeled preparation did not show immunogold labeling (Figure C)

IC 100 disrupts ASC filament architecture and function, as determined by polymerization of purified ASC^{PYD} for 20 – 30 minutes, followed by micrographs taken at various time points with and without IC 100 as depicted below.

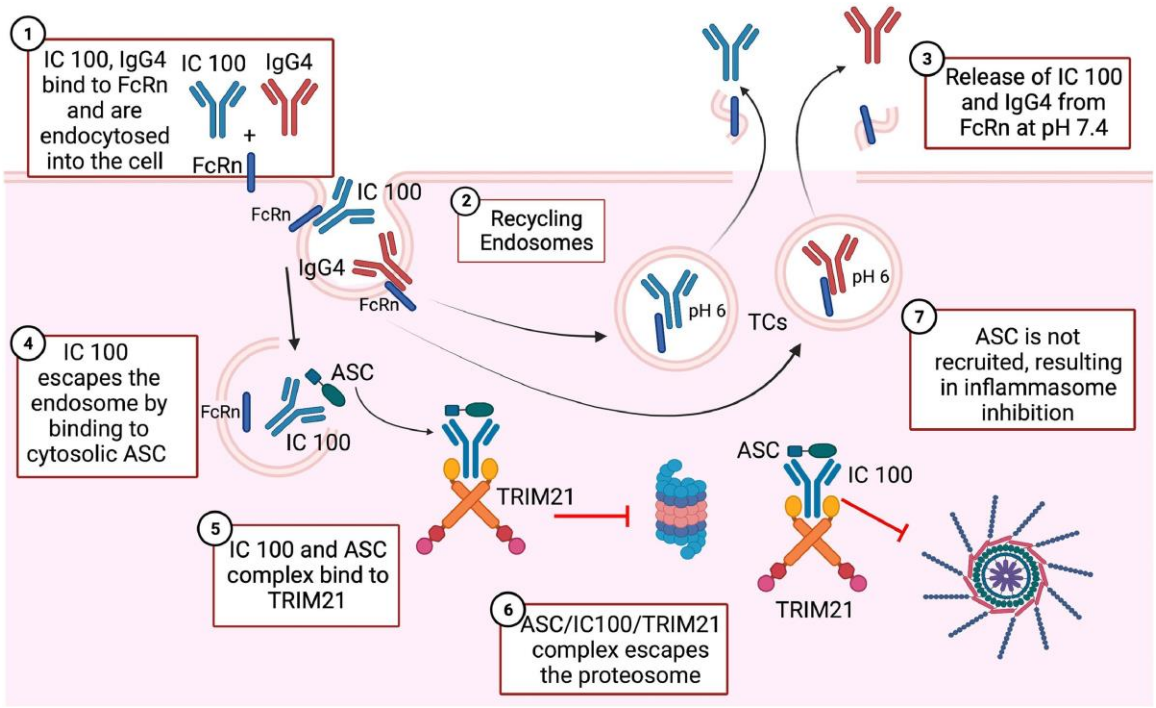
IC 100 Binds to ASC^{PYD} Filaments



- Although IC 100 did not prevent filament growth (Figure A, right), it disrupted the architecture of the filaments formed (Figure E
- The filaments appeared to be clumped into bundles with dark interspersed deposits (Figures B and C, arrows)
- Electron micrographs of IC 100 labeled ASC^{PYD} filaments showed that these dark deposits w predominantly located at the ends of the filaments, where the ASC^{PYD} binding site is mo accessible (Figures B and C, arrows)

Schematic of IC 100 Intracellular Mechanism of Action[3]

Following is a schematic illustration of the intracellular mechanism of action of IC 100.



(1) IC 100 and IgG4 are internalized into cells by fluid-phase pinocytosis into early endosomes; (2) Once early endosomes are acidified (pH 6.0), the affinity between IC 00/IgG4 and FcRn increases, resulting in binding; (3) Some of the FcRn-IC 100 and FcRn-IgG4 complexes are sorted into tubulovesicular transport carriers (TCs), which recycle to the plasma membrane and undergo exocytosis. The physiological pH in the extracellular space reduces the affinity of FcRn for IC 100 andIgG4, returning the immunoglobulins to the extracellular environment; (4) Some IC 100 escapes the endosome by binding with intracellular ASC; (5) ASC-IC 100 binds to TRIM21, forming a tripartite complex; (6) The ASC/IC 100/TRIM21complex is not directed for proteosomal degradation but rather remains in the intracellular compartment for at least 3 days; (7) Thus preventing intracellular ASC from recruitment and assembly into the inflammasome

IC 100 Pharmacology

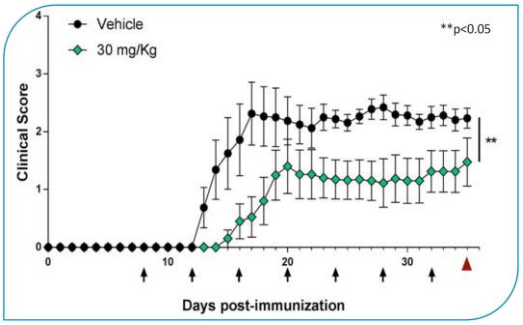
Multiple Sclerosis (MS)[4]

MS, which affects around 1 million people in the US and 2.8 million people worldwide, is an autoimmune demyelinating disease of the central nervous system characterized by an inflammatory response sustained by innate and adaptive immune mechanisms dependent on lymphocyte (both T and B cells) and myeloid cell activation. To determine the potential of Inflammasome ASC Inhibitor IC 100 for treatment of MS, IC 100 was administered IP to EAE-

induced mice at 10, 30, or 45 mg/kg on day 8 before appearance of clinical symptoms, followed by treatment every 4 days for 32 days.

The optimal dose of IC 100 in the EAE model was 30 mg/kg. At this dose, IC 100 delayed disease onset and significantly improved functionality, based on MS clinical scores depicted below.

MS Clinical Scores in EAE Mice

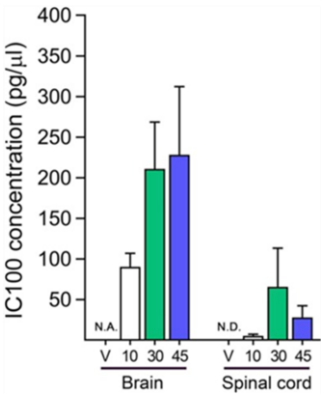


MS Clinical Scoring

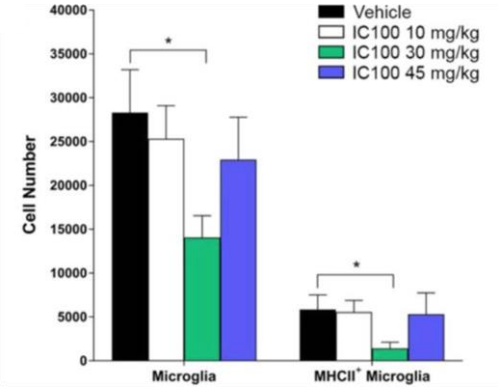
1 = Loss of tail tonicity
2 = Mild hind limb weakness
3 = Partial hind limb paralysis
4 = Complete hind limb paralysis
5 = Complete hind limb paralysis with forelimb weakness or morbidity

At the optimal dose of 30 mg/kg, IC 100 penetrated the spinal cord and decreased the number of activated microglial, CD4+, CD8+, and myeloid cells in the EAE model as depicted below.

IC 100 CNS Penetration

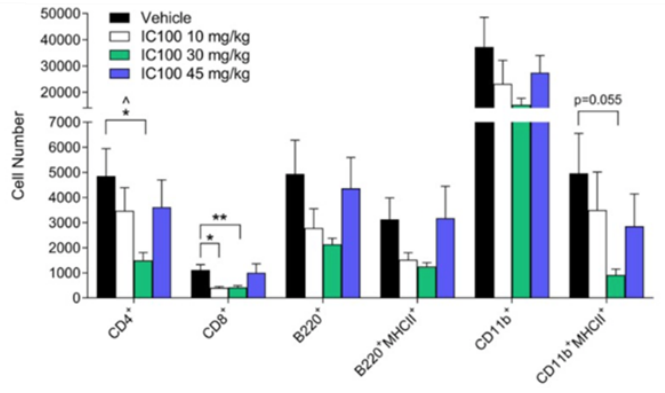


Spinal Cord
Activated Microglial Cells



*p<0.05

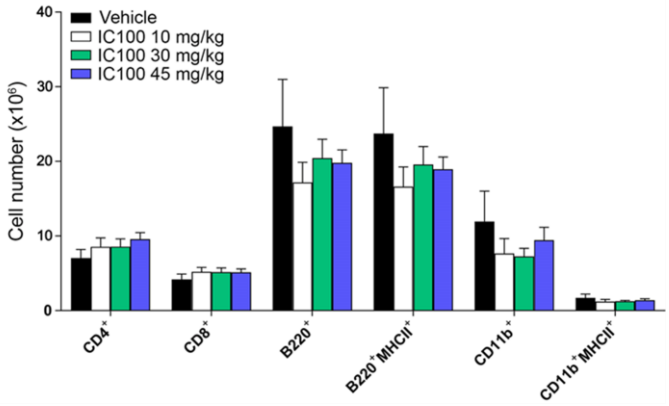
Spinal Cord Infiltration
of Peripheral Immune Cells



* p<0.05; ^ p<0.05; ** p<0.001

IC 100 did not affect splenic immune cell populations in the EAE mouse model, as depicted below, indicating that the ability to mount an adequate immune response was preserved.

Splenic Immune Cell Populations



This study indicates that IC 100 attenuates the immune-inflammatory response that drives EAE development and progression without broad immunosuppression, thereby identifying ASC as a promising target for the treatment of MS and other neurological diseases with a neuroinflammatory component.

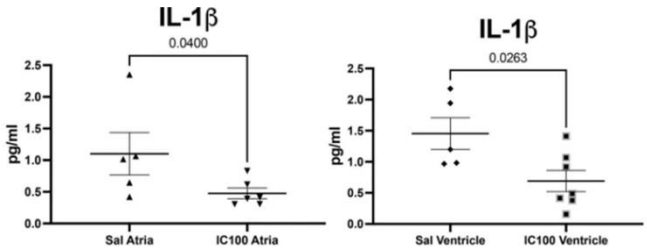
Stroke-related Cardiovascular Injury and Dysfunction[5]

Cardiac complications are among the leading causes of mortality and morbidity following a cerebrovascular stroke, which affects 795,000 people annually in the US. Nearly 70% of patients with acute ischemic stroke have acute cardiac dysfunction such as arrhythmias, electrocardiogram changes, and myocardial ischemic-like conditions, and these complications

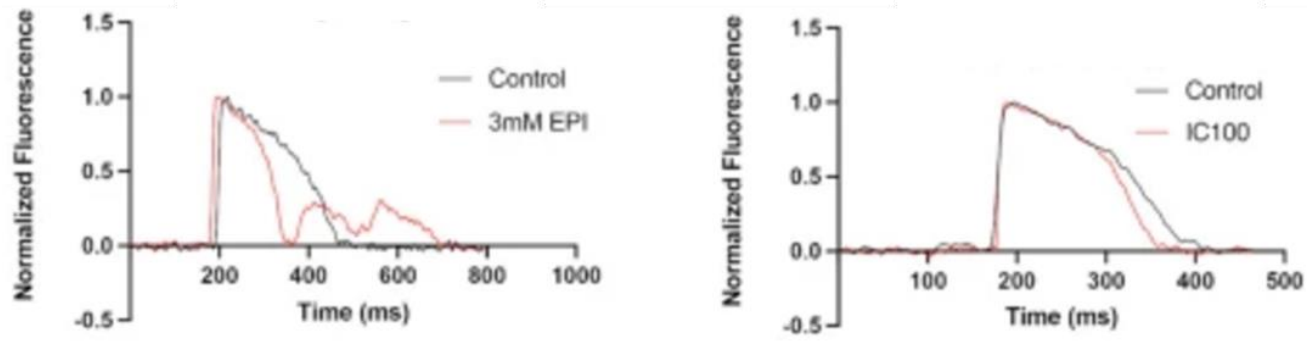
account for over 1.5 million deaths worldwide. The pathomechanisms underlying post-stroke cardiac dysfunction include a surge of catecholamines and an inflammasome-induced systemic and cardiac inflammatory response. A mouse model of photothrombotic stroke (PTS) was used to determine if Inflammasome ASC Inhibitor IC 100 can attenuate post-stroke cardiac inflammation. Additionally, catecholamine-treated zebrafish hearts were used to determine if IC 100 can protect against post-stroke cardiac dysfunction. IC 100 was administered IV at 30 mg/kg to mice thirty minutes post-PTS. Action potential duration was evaluated in excised zebrafish hearts with and without IC 100 (10 µg/ml) 20 seconds after catecholamine treatment.

IC 100 attenuated cardiac inflammation post-stroke as evidenced by significant reductions in IL-1β, and it improved cardiac function as evidenced by attenuation of the shortened action potential duration depicted in the images below.

IL-1β Levels in Mouse Atria and Ventricles



Ventricular Action Potential Traces from Excised Zebra Fish Hearts Exposed to Epinephrine



These data demonstrate the potential for IC 100 to attenuate stroke-related cardiovascular disease.

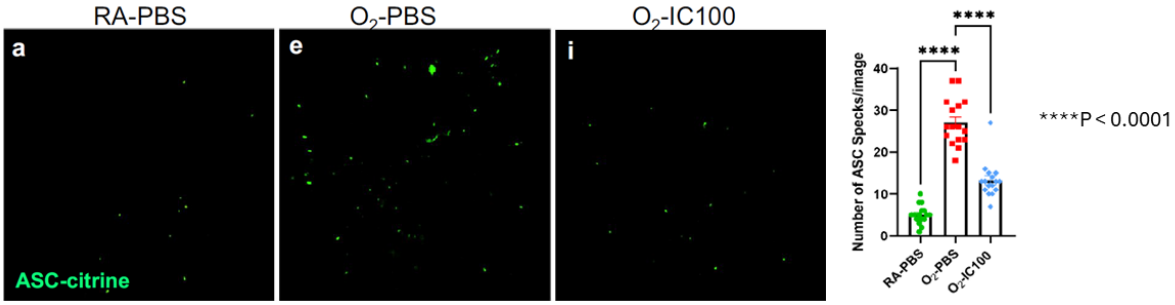
Retinopathy of Prematurity[6]

Retinopathy of prematurity (ROP) affects around 60% of very low-birthweight infants needing oxygen therapy, and it is the leading cause of childhood vision impairment and blindness worldwide. The hallmarks of ROP are characterized in two phases, delayed vascular development in phase 1 and intravitreal neovascularization in phase 2. ASC speck-induced

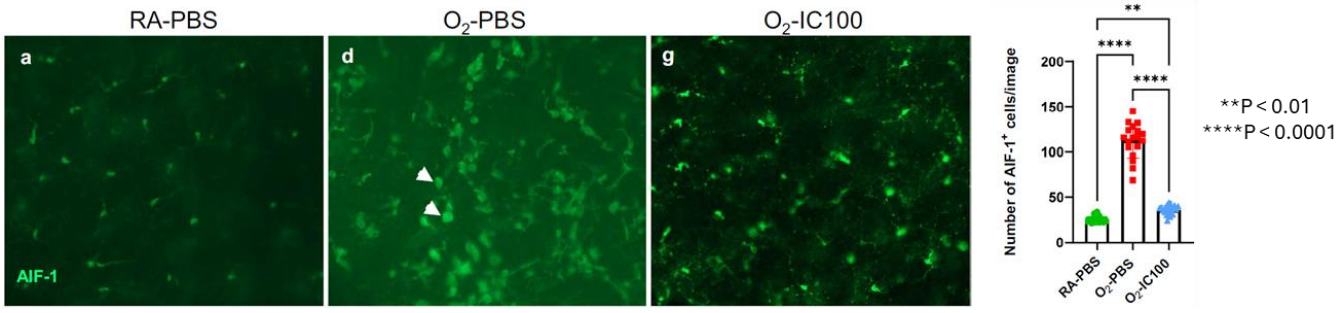
retinal microglia activation leading to inflammation and pyroptosis have been shown to contribute to development of ROP. Oxygen-induced mouse models of retinopathy (OIR models) were evaluated to determine if Inflammasome ASC Inhibitor IC 100 has potential to attenuate retinal microglial activation and inflammation leading to retinopathy and impaired vision. IC 100 was administered immediately after 5 days of 75% O2 exposure by multiple IP injections of 10 or 20 µg/g or by single IVT injection of 2.5 µg/0.5 µL per eye.

IC 100 attenuated retinal inflammation as evidenced by a 55% reduction in retinal ASC speck formation and reduced microglial density and activation compared to the placebo-treated oxygen-exposed retina (0_2-PBS), as depicted below.

Retinal ASC Speck Formation

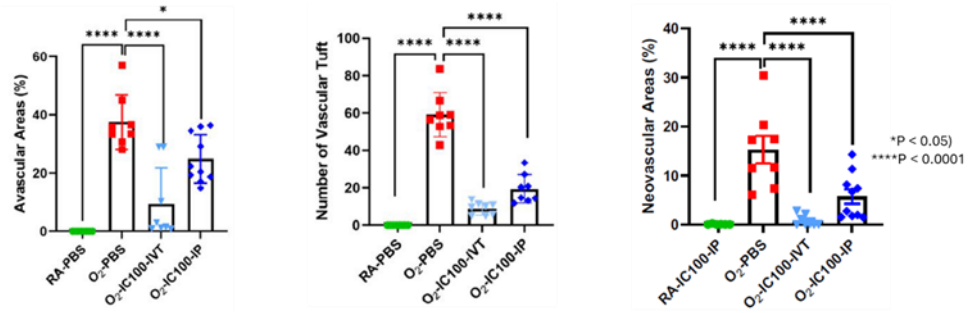


Retinal Microglial Density and Activation



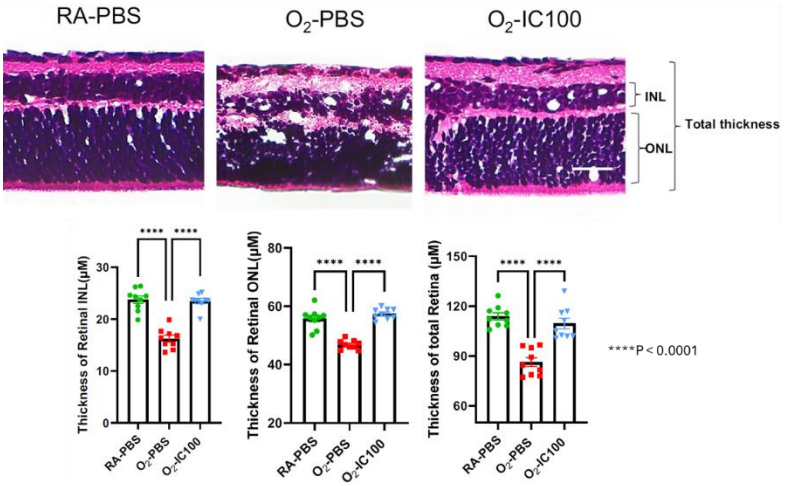
IC 100 alleviated vaso-obliteration. Compared to the placebo-treated oxygen-exposed retina (0_2-PBS), IC 100 reduced the percentage of avascular areas, the number of vascular tufts, and the percentage of intravitreal neovascularization, as depicted below.

Quantification of Avascular Areas, Vascular Tufts, and Neovascular Areas

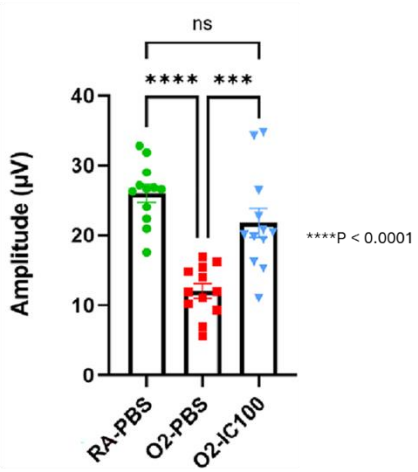


IC 100 restored retinal structure. With IC 100, the thickness of the inner nuclear layer (INL), outer nuclear layer (ONL), and total retinal layer was comparable to the placebo control (RA-PBS). IC 100 also restored retinal function. With IC 100 there was a 70% increase in amplitude compared to the placebo-treated oxygen-exposed retina (0_2-PBS). Results are depicted below.

Retinal Layer Thickness



Amplitude

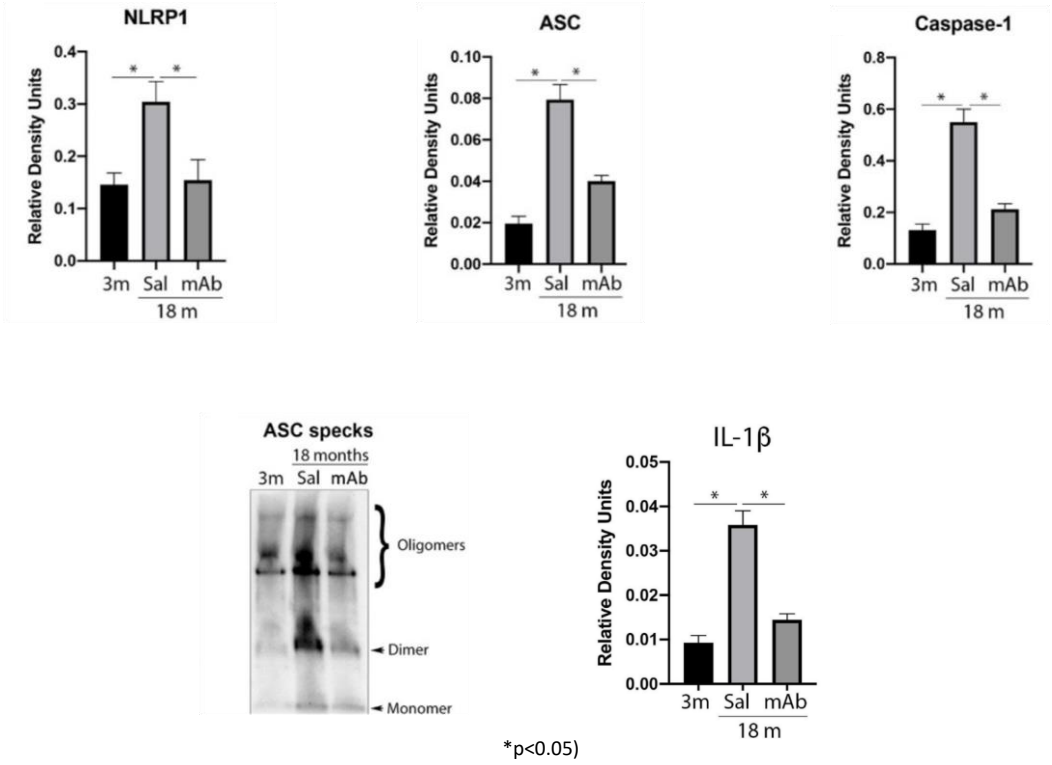


These data demonstrate that inhibition of ASC speck formation by IC 100 in an animal model of retinopathy of prematurity reduced retinal inflammation and its resulting structural damage and disfunction.

Age-related Inflammation[7]

Age-related chronic inflammation, known as inflammaging, is a risk factor for neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. To determine the potential of Inflammasome ASC Inhibitor IC 100 to modulate age-related inflammation in the brain, male mice, aged 3 and 18 months old, were treated with IC 100 or saline intraperitoneally at 10 mg/kg and sacrificed 3 days later for analysis of their brain cortex.

IC 100 significantly reduced cortical inflammasome proteins (NLRP1, ASC, Caspase-1), ASC Specks, and inflammation (IL-1β), as depicted below.



*p<0.05)

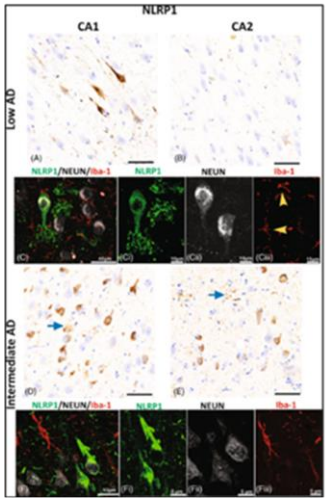
Data support that targeting ASC specks may have potential to ameliorate age-related inflammation associated with neurodegenerative diseases.

Alzheimer's Disease (AD)[8]

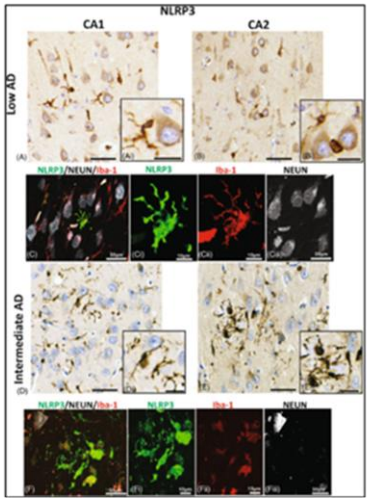
AD is a progressive neurodegenerative disease affecting 6.7 million people in the US, with an estimated 500,00 new cases annually. AD destroys memory and cognitive function. The pathomechanisms that contribute to AD include chronic neuroinflammation, accumulation of misfolded protein aggregates of extracellular amyloid-β (Aβ), intracellular hyperphosphorylated tau (pTau), and neurofibrillary tangles. To determine the role of inflammasome activation in AD and its progression, a panel of commercially available antibodies were used to identify Aβ and pTau, and inflammasome proteins NLRP1, NLRP3, and caspase-1 were measured in postmortem human brains with and without intermediate AD neuropathological changes. Additionally, IC 100, which targets ASC^{PYD} and a commercially available anti-ASC antibody targeting ASC^{card} were used to determine the cell-type distribution of ASC in postmortem brains from donors with AD.

Expression of inflammasome proteins NLRP1, NLRP3, ASC, and caspase-1 occurred early in AD, indicating a role for multiple types of inflammasomes in disease development. NLRP1 was expressed primarily in neurons and NLRP3 was expressed primarily in microglia as depicted below.

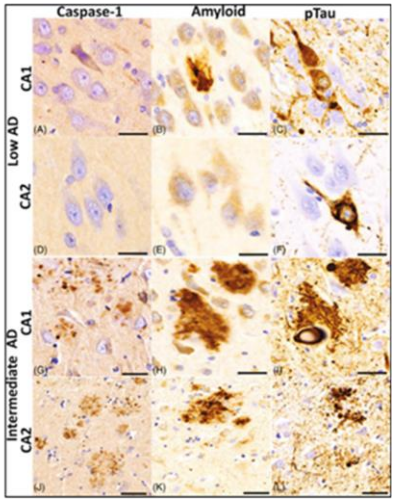
NLRP1 in Hippocampal Neurons



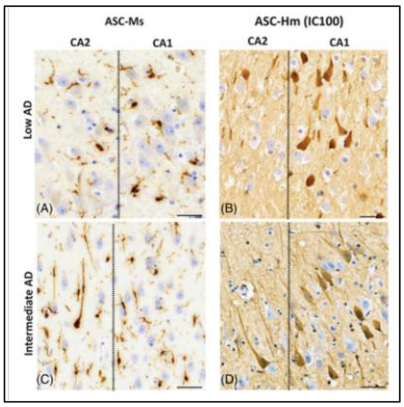
NLRP3 in Microglia



Caspase-1 in Hippocampal Regions



ASC in Neurons & Microglia



This study demonstrates that increased expression of multiple types of inflammasomes (NLRP1 and NLRP3) occurs early in AD, and that IC 100 binds to neuronal ASC. This supports the potential of Inflammasome ASC Inhibitor IC 100 as a diagnostic and therapeutic option for early intervention to potentially delay AD progression.

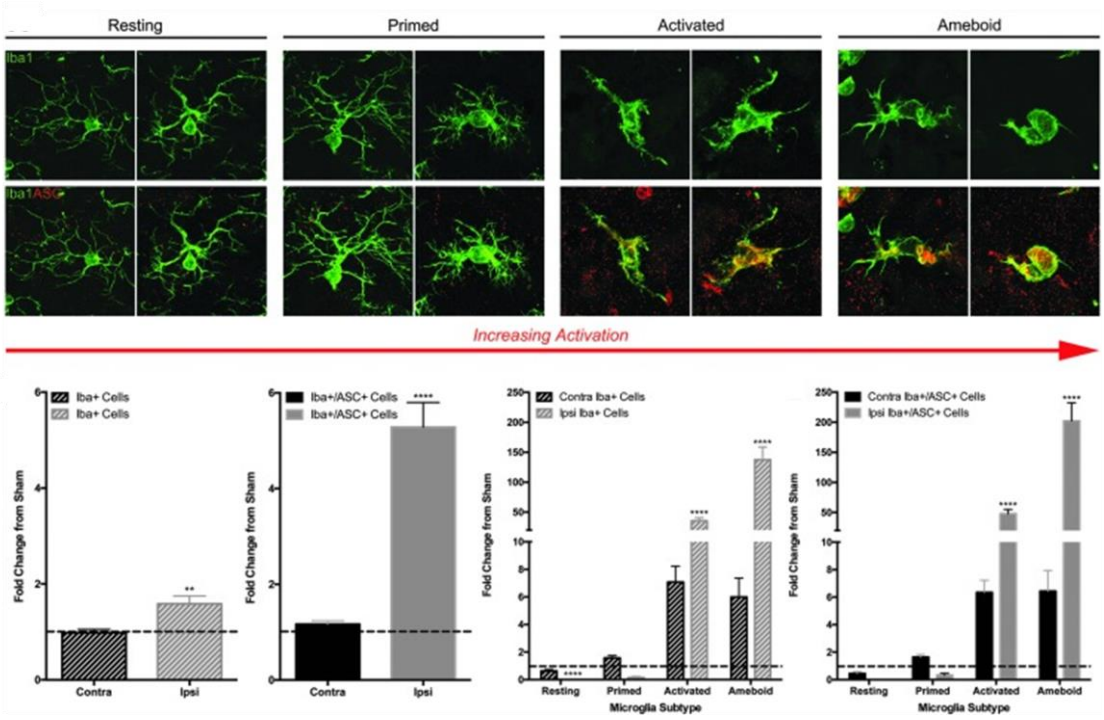
Penetrating Traumatic Brain Injury (PTBI)[9,10]

PTBI is the most severe form of traumatic brain injuries, and a significant cause of death, predominantly due to firearm injuries. In the United States, approximately 20,000 gunshot

injuries to the head occur annually. Additionally, PTBI survivors often suffer severe neurological outcomes, such as persistent vegetative state or severe disability associated with the primary mechanical injury, which is magnified by secondary injury from inflammation among other things. To determine the contribution of inflammasome signaling after PTBI and the effects of Inflammasome ASC Inhibitor IC 100 on inflammasome activation and pyroptosis, 100 adult male Sprague-Dawley rats were subjected to sham procedures or penetrating ballistic-like brain injury (PBBI) and administered IC 100 IV at 5 mg/kg four hours after injury.

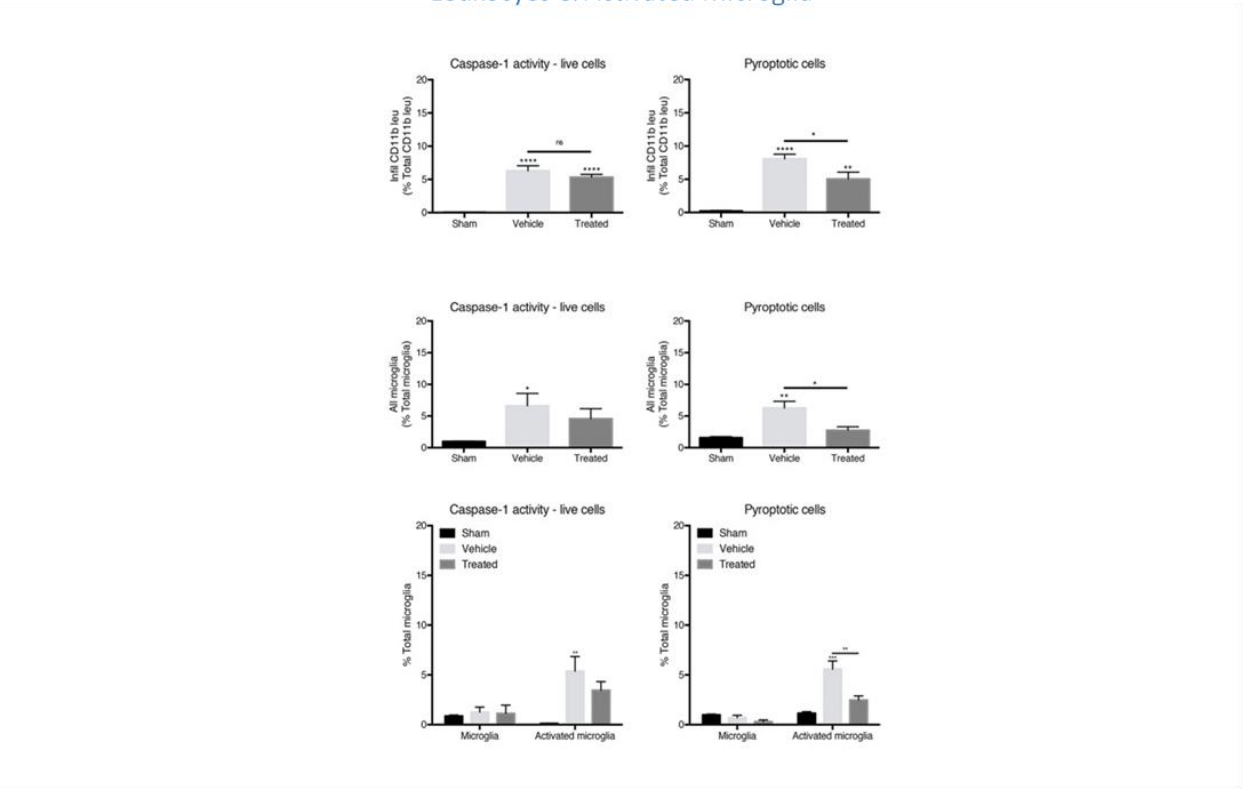
Inflammasomes were activated in microglia 48 hours following PBBI, as evidenced by the significant increase in number of activated and ameboid type microglia expressing ASC in the injured cortex depicted below. Inflammasome activation persisted for 12 weeks following injury.

Activated and Ameboid Microglia Expressing ASC



Caspase-1 activity and pyroptosis were also increased in activated microglia and infiltrating leukocytes 48 hours after PBBT, both of which were reduced by treatment with IC 100 as depicted below.

Caspase-1 Activity & Pyroptosis in Infiltrating Leukocyes & Activated Microglia



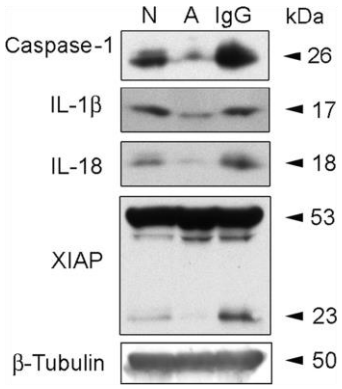
Data show that the neuro-inflammatory response to PTBI includes pyroptotic cell death mediated by inflammasome activation, leading to continued tissue loss in surrounding brain regions that can be attenuated by IC 100.

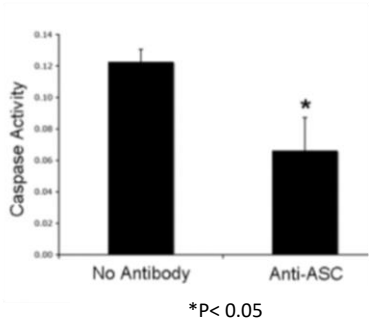
Spinal Cord Injury (SCI)[11]

In the United States, there are approximately 17,000 new cases of SCI each year, and roughly 282,000 persons are estimated to be living with SCI. SCI is an insult to the spinal cord resulting in a change in the cord's normal motor, sensory, or autonomic function. A vigorous immune response mediated by inflammasome activation is induced by the injury, producing a secondary injury cascade. Patients with SCI usually have permanent and often devastating neurologic deficits and disability. To determine the effects of inflammasome ASC inhibition on inflammasome activation and histopathological and functional outcomes, female Fischer rats were subjected to moderate cervical SCI, and an anti-ASC tool antibody (50 µg) was injected IV or IP 20 min after SCI for up to 7 days depending on the evaluation. Animals were sacrificed 24 hours after the last treatment. Controls were treated with an equal amount of IgG and saline or were left untreated.

Inflammasome ASC inhibition reduced inflammasome activation as evidence by decreased levels of caspase-1, IL-1β and IL-18, and XIAP depicted below.

Inflammasome Proteins

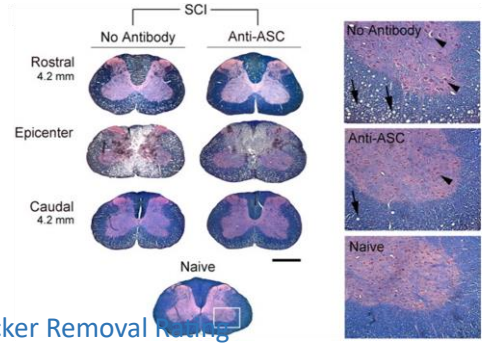


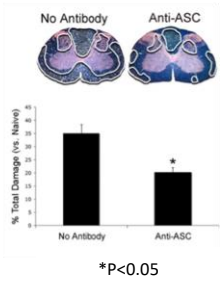


*P< 0.05

Inflammasome ASC inhibition improved histopathological outcomes and decreased spinal cord lesion volume as depicted below.

Histopathology

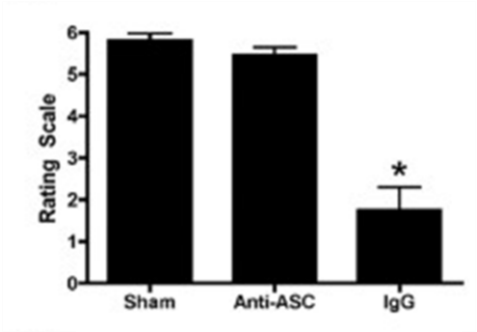




*P<0.05

as

Sticker Removal Rating



P<0.05

Sticker Removal Rating Scale

1 = No sticker removal attempted
2 = Forelimbs did not reach sticker
3 = Forelimbs reach sticker after head was contracted, but sticker not removed
4 = Forelimbs reach sticker, but sticker not removed
5 = Sticker removed after several attempts
6 = Sticker removed with no difficulty

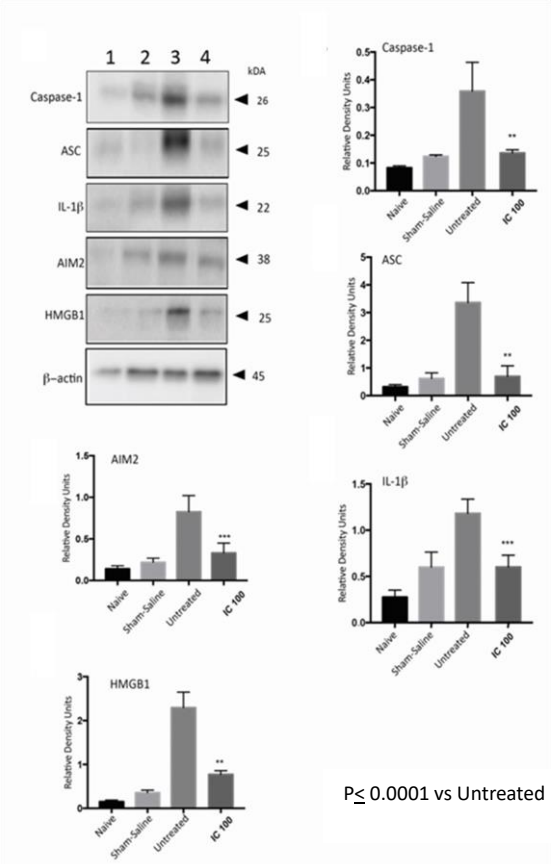
This study demonstrates that inflammasome ASC inhibition significantly reduced inflammasome activation in SCI, resulting in significant improvements in tissue sparing and functional recovery.

Acute Lung Injury ALI/Acute Respiratory Distress Syndrome (ARDS)[12]

ALI and ARDS, the most severe form of ALI, describe clinical syndromes of acute respiratory failure associated with rapid onset of widespread inflammation in the lungs. ALI, which affects around 200,000 people annually in the US, is associated with substantial morbidity and mortality. There is evidence that long-term quality of life is adversely affected even in patients who survive ALI. ALI occurs in around 20–25% of traumatic brain injury (TBI) patients. Inflammasomes play a critical role in TBI-induced secondary pathophysiology, such as ALI. Following TBI, inflammasome proteins and bioactive cytokines such as IL-1β a are released into the peripheral circulation in extracellular vesicles (EV). EV-mediated inflammasome signaling can trigger an immune response and amplify inflammation in other organs, such as the lungs. To determine the impact of Inflammasome ASC Inhibitor IC 100 on inflammasome activation in the lungs triggered by TBI, ALI and subsequent ARDS was induced by delivering extracellular vesicles (EV) from mice with traumatic brain injury into naïve mice, followed by IV administration of IC 100 at 5 mg/kg one hour after EV delivery. Animals were sacrificed 24 later for evaluation.

IC 100 significantly reduced inflammasome expression in lungs of animals treated with EV from TBI-injured mice, as evidenced by a reduction in AIM2, caspase-1, ASC, IL-1β, and HMGB1 depicted below.

Inflammasome Proteins



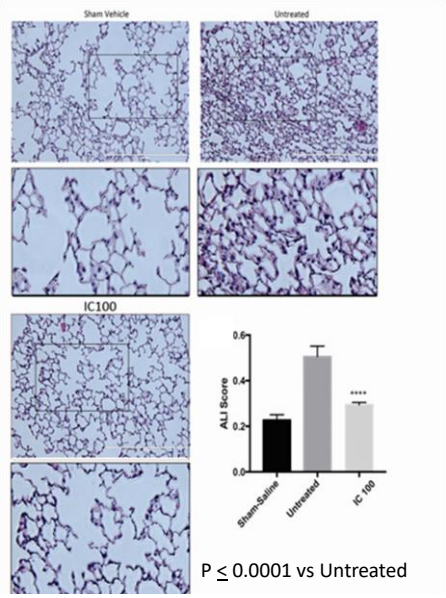
P≤ 0.0001 vs Untreated

Immunoblot Legend

1 = Naïve
2 = Sham-Saline
3 = Untreated
4 = IC 100

IC 100 improved histopathological outcomes in lungs of animals treated with EV from TBI-injured mice, as evidenced by significantly less neutrophil infiltration into alveolar and interstitial space, and improved ALI scores as depicted below.

Histopathology



These data demonstrate that C 100 inhibited inflammasome activation in lung tissue and prevented development of ALI from EV-mediated inflammasome signaling associated with TBI.

IC 100 Safety

To assess the preliminary safety of Inflammasome ASC Inhibitor IC 100, dose-ranging toxicology studies were conducted in rodents (CD-1 strain mice) and non-human primates (cynomolgus monkeys).

Mouse Dose-ranging Toxicology

To characterize the toxicity and toxicokinetic profiles of IC 100, a repeat-dose 21-day study was conducted in CD-1 strain mice. Mice received weekly IV doses of IC 100 at 30, 100, or 300 mg/kg for 3 weeks. Toxicokinetics were assessed following a single dose and after three weekly doses. End-of-study samples were obtained for ADA evaluation.

Results are depicted below.

Parameter	Results
Body Weight	No drug-related changes
Clinical Observations	No drug-related observations
Clinical Pathology	• Clin chem: minimal to mild increases in ALP, CK, TRIG, GLOB, A/G, & PHOS; not considered adverse given the small magnitude of the changes, lack of dose- response relationship, and lack of confirmatory microscopic findings • Hematology: mild increases/decreases in LYMPH and MONO resulting in minimally increased/decreased WBC in affected groups; not considered adverse given opposite trends in males/females and non-dose relationship
Macroscopic Observations	No drug-related observations
Microscopic Observations	No drug-related observations
Mortality	No drug-related unscheduled deaths
Toxicokinetic Parameters	• Tmax within 0.5 hour • Half-life: 8 to 14 days • Systemic exposure increased with increasing dose levels • Similar exposure between sexes
ADA	**No anti-IC 100 antibodies detected**

This study showed that IV administration of IC 100 once weekly for 3 weeks in mice was well tolerated at dose levels up to 300 mg/kg/week. The systemic exposure of IC 100 increased with increasing dose, with slight gender effects observed. Although the data is limited, half-life was 8 - 14 days, consistent with previous data and supports the increased exposure in high dose animals evaluated for toxicological effects.

There were no IC 100 related mortalities, changes in body weight, clinical observations, macroscopic or microscopic observations. Observed changes in clinical pathology were considered non-adverse given the low magnitude of changes and the lack of true dose-relationship. The no-observed-adverse-effect level (NOAEL) was 300 mg/kg/dose. The systemic exposure to IC 100 increased with increasing dose levels for both genders.

NHP Dose-ranging Toxicology

To characterize the toxicity and toxicokinetic profiles of IC 100, a repeat-dose 21-day study was conducted in cynomolgus monkeys. Monkeys received weekly IV dosing of IC 100 at 30, 100, or 300 mg/kg for 3 weeks. Toxicokinetics were assessed following a single dose and after three weekly doses. End-of-study samples were obtained for ADA evaluation. TK data are not yet available.

Results are depicted below.

Parameter	Results
Food Consumption	No drug-related findings
Body Weight	No drug-related findings
Clinical Observations	No drug-related observations
Clinical Pathology	• Clinical chemistry: No drug-related findings • Hematology: No drug-related findings • Clotting: No drug-related findings
ECG	No drug-related changes
Macroscopic Observations	No drug-related findings day 22; mottled discolored livers day 45 in one of one mid-dose female, one of one each high dose male & Female; liver discoloration an artifactual change
Microscopic Observations	No drug-related findings day 22 (full tissue list); No drug-related findings in liver on day 45 (only tissue examined)
Mortality	No drug-related mortality
Toxicokinetic Parameters	Not yet available
ADA	**No anti-IC 100 antibodies detected**

No gross or histopathologic changes were observed in animals receiving 300 mg/kg/dose that were euthanized on Study Day 22. In animals euthanized on Study Day 45, a gross finding of mottled discoloration was noted in the liver of females receiving the 100 mg/kg/dose and in animals receiving the 300 mg/kg/dose of IC 100. This is commonly observed in this species, and there were no histopathologic correlates. The NOEL was 300 mg/kg/dose. Toxicokinetic analysis is pending qualification of analytical methods.

Summary and Conclusions

Inflammasomes are intracellular multiprotein complexes of the innate immune system that initiate inflammation in response to either exogenous pathogens and viruses or endogenous danger signals. Inflammation provides protection by eliminating pathogens and promoting tissue repair and recovery. A normal inflammatory response is temporary and resolves once the threat has passed. Shifts in the inflammatory response from short- to long-term can lead to chronic inflammatory diseases, which have been recognized as the most significant cause of death in the world today.

Inflammasome ASC has potential as an important therapeutic target based on the following:

- Has a critical role in initiation, amplification, and perpetuation of the inflammatory response,
- Is central to formation and activation of different inflammasome complexes, associated with up to 12 sensor molecules, with potential to better control inflammation in

diseases and conditions associated with activation of more than one inflammasome complex

- Inhibition will block inflammasome formation to stop initiation of the inflammatory cascade, and will disrupt the structure and function of ASC specks, intracellularly and extracellularly, blocking perpetuation of systemic inflammation

Inflammasome ASC Inhibitor IC 100 was developed to address heightened and prolonged inflammasome activation that perpetuates damaging inflammation underlying numerous CNS and other inflammatory conditions. It is a humanized monoclonal IgG4 antibody that binds to the PYD domain of adaptor ASC to block PYD-PYD molecular interactions across multiple inflammasome complexes.

Inflammasome ASC inhibitor IC 100 has potential as a promising therapeutic option for numerous inflammatory diseases based on the pre-clinical data presented in this paper and summarized below.

- By inhibiting ASC, IC 100 blocks both initiation and perpetuation of inflammation
- MOA has been validated in pre-clinical studies for multiple indications: multiple sclerosis, stroke-related cardiovascular injury and dysfunction, retinopathy of prematurity, age-related inflammation/early Alzheimer's disease, traumatic brain injury, and acute respiratory distress syndrome
- Promising safety profile
  - Attenuates inflammation without broad immunosuppression
  - Lower immunogenicity (9%) than many biologics
  - No drug-related AEs or histopathology changes at weekly doses up to 300 mg/kg for 21 days in dose-ranging toxicology studies (mice & NHP)

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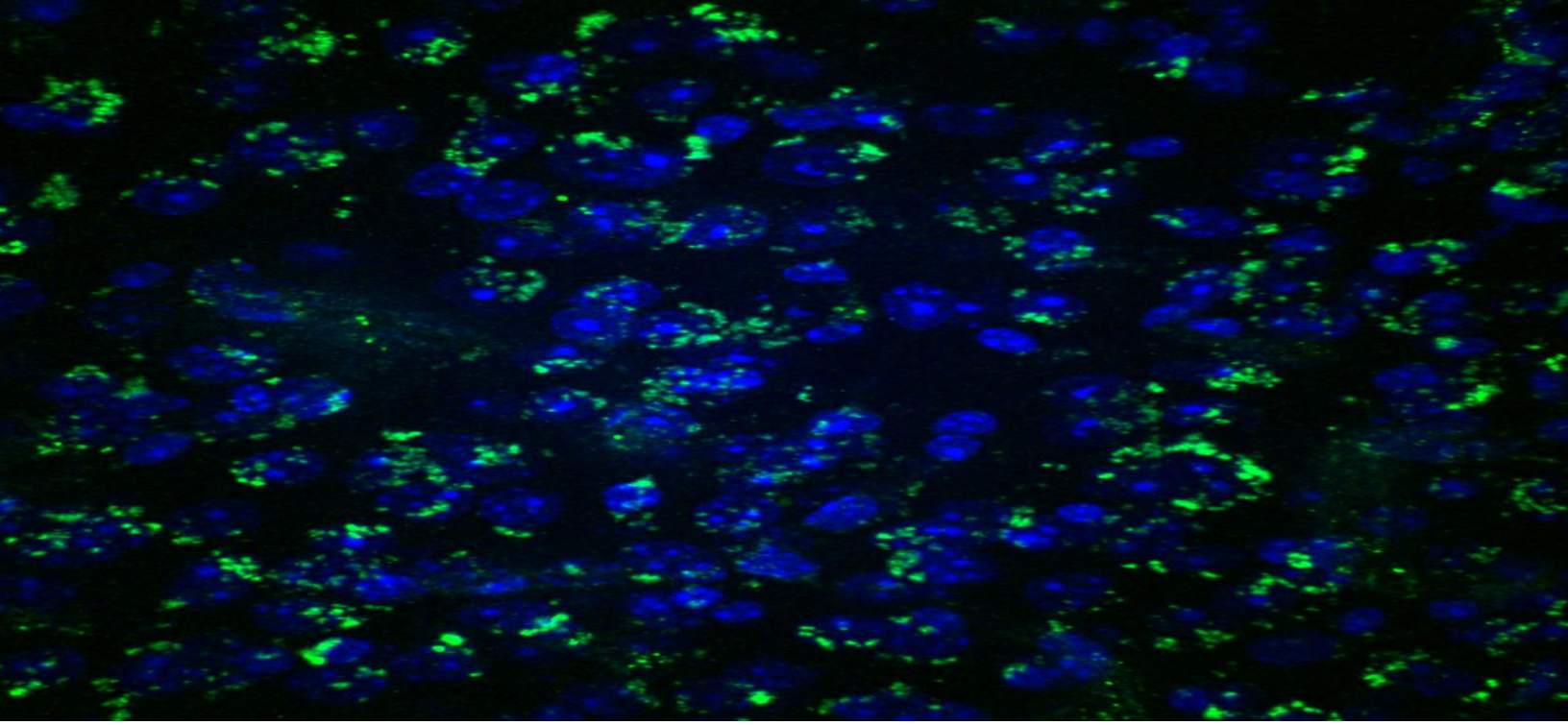

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