Inflammasome ASC Inhibitor IC 100, Promising Therapeutic Potential For Neurological Diseases

Background

Up to 16 million people over 18 years old in the US are living with a neurological disease/disorder, and 1.2 million new cases are diagnosed annually. Neurological diseases, the leading cause of physical and cognitive disability, typically involve functional impairment or loss of neurons leading to neurological dysfunction. Inflammasome-induced inflammation is a key factor in the development and progression of a variety of neurological diseases, including immunological diseases such as multiple sclerosis; neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases; and brain insults, such as stroke and traumatic brain injury.[1]

Accumulating data suggest that activation of more than one type of inflammasome contributes to the pathogenesis of many neurological diseases, as illustrated in the table below.

Disease	Potential Inflammasomes Involved
Alzheimer's Disease[1,7]	NLRP1, NLRP2, NLRP3
Parkinson's Disease[1]	NLRP3, AIM2
Amyotrophic Lateral Sclerosis[1]	NLRP1, NLRP3, NLRC4, AIM2
Multiple Sclerosis[1]	NLRP1, NLRP3, NLRC4, AIM2
Stroke[1]	NLRP1, NLRP2, NLRP3, NLRP6, NLRP10, AIM2
Traumatic Brain Injury[1]	NLRP1, NLRP3, NLRC4, AIM2

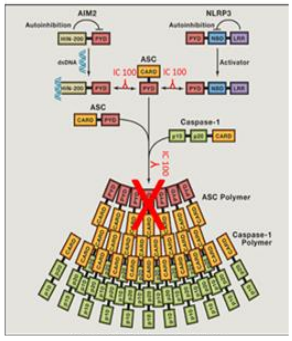
Given the currently limited data on the specific types and numbers of inflammasome pathways involved in the development and progression of any one neurological disease, Ravichandran and Heneka suggested that targeting common

downstream effectors, such as ASC, could be an effective way of preventing inflammatory reactions, regardless of the specific activated inflammasome.[1]

A growing body of evidence supports the role of extracellular ASC specks and ASC in the progression of neurological diseases, as summarized below.

- In a mouse study of Alzheimer's disease (AD), extracellular ASC specks in the brain acted as a molecular scaffold to initiate Aβ aggregation and accelerate formation of Aβ plaques, leading to an increased Aβ plaque burden and neurodegeneration.[1]
- In postmortem cases of people with AD pathology, recruitment of ASC molecules and formation of inflammasome complexes in both neurons and microglia led to loss of plasticity and neuronal scaffolding proteins, which leads to memory and learning deficits.[2]
- In a mouse study of Parkinson's disease (PD), there was a clear association between ASC speck assembly, NLRP3 inflammasome activation, and propagation of α-synuclein pathology.[3]
- Another mouse study of PD demonstrated that co-injection of α-synuclein preformed fibrils with ASC specks increased levels of inflammasome components in the mid-brain and worsened motor function.[1]
- Knockout of ASC in an EAE mouse model of multiple sclerosis provided protection against development of MS.[1]
- Injection of recombinant ASC into a mouse model of stroke worsened outcomes, indicating that the inflammasomes involved in stroke influence outcomes via an ASC-mediated inflammatory response.[1]

Inflammasome ASC Inhibitor IC 100



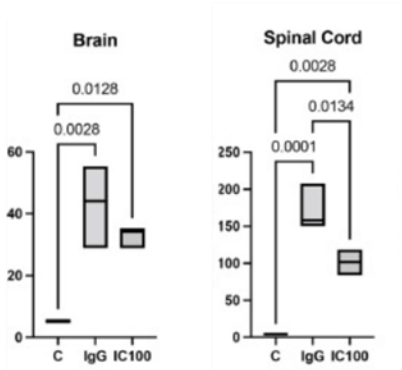
IC 100 is in development to ameliorate heightened and prolonged inflammasome activation that perpetuates damaging inflammation underlying numerous CNS and peripheral inflammatory conditions. IC 100 is a humanized monoclonal IgG4 antibody that binds to the PYD domain of adaptor ASC to block PYD-PYD molecular interactions across multiple types of inflammasomes, not just NLRP3. This is expected to improve control of inflammation in numerous diseases associated with activation of more than one type of inflammasome. By blocking PYD-PYD interactions, IC 100 prevents ASC binding to

sensor proteins to inhibit inflammasome formation, and thus block initiation of the inflammatory cascade. IC 100 also disrupts intracellular and extracellular formation of ASC specks to stop the spread and perpetuation of damaging inflammation.[4]

Based on ZyVersa's preclinical program, Inflammasome ASC Inhibitor IC 100 shows promising therapeutic potential for treatment of neurological diseases. Data demonstrate that IC 100 crosses the blood brain barrier and penetrates the brain and spinal cord. Likewise, IC 100 demonstrates strong pharmacological signals in animal models representative of a variety of neurological conditions: multiple sclerosis, age-related inflammation, Alzheimer's disease, traumatic brain injury, and spinal cord injury. Work is currently underway in Parkinson's disease through a grant with the Michael J. Fox Foundation. Following is an overview of the preclinical data collected to date with IC 100.

CNS Distribution

IC 100 demonstrated significantly higher distribution in the brain and spinal cord than controls, based on fluorescent labeling and in vivo imaging in B6 albino mice.[4]



Units: Average radiant efficiency
(photons/s/cm2/sr/µW/cm2); mean/+/- SEM

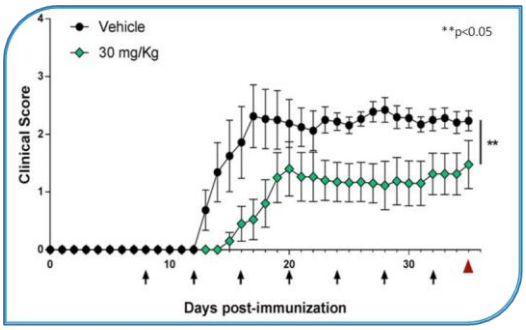
IC 100 Pharmacology

Multiple Sclerosis (MS)[5]

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 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 was 30 mg/kg. At this dose, IC 100 delayed disease onset and significantly improved functionality, based on MS clinical scores.

MS Clinical Scores in EAE Mice

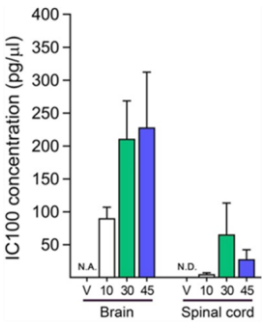


MS Clinical Scoring

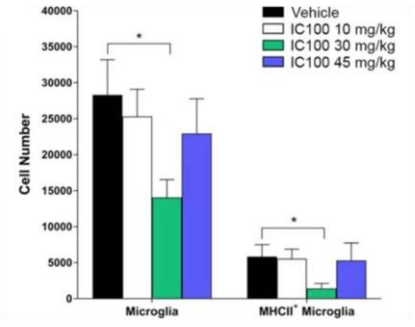
1 = Loss of tail tonicity
2 = Mild hind limb weakness
3 = Partial hind limb paralysis
4 = Complete hind limb paralysis
5 = Complete hind limb paralysis with forelimb weakness or morbidity

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.

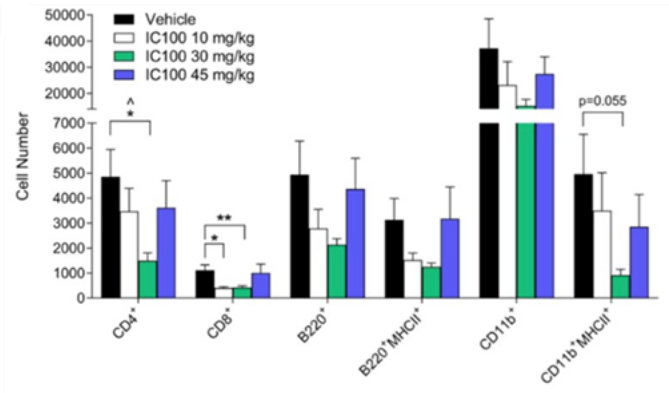
IC 100 CNS Penetration



Spinal Cord
Activated Microglial Cells



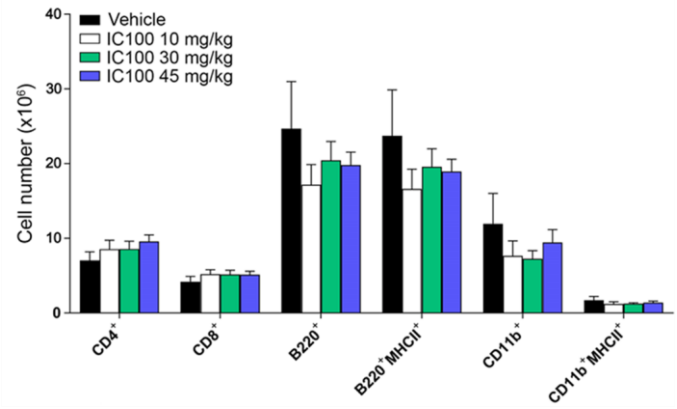
Spinal Cord Infiltration
of Peripheral Immune Cells



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

IC 100 did not affect splenic immune cell populations, indicating that the ability to mount an adequate immune response was preserved.

Splenic Immune Cell Populations



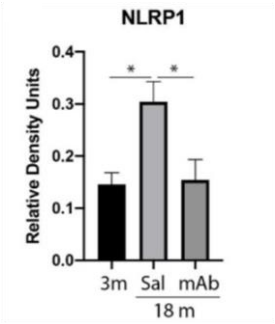
This study demonstrated that IC 100 attenuates the immune-inflammatory response that drives EAE development and progression, without broad immunosuppression, thereby identifying ASC as a promising target for the treatment of MS and other neurological diseases with a neuroinflammatory component.

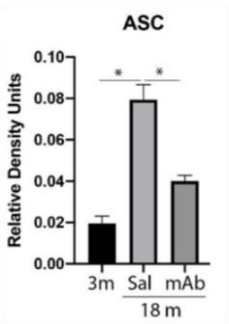
Age-related Inflammation[6]

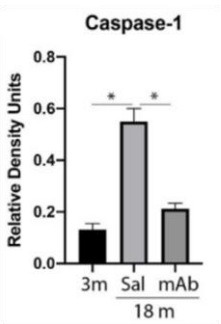
Age-related chronic inflammation, known as inflammaging, is a risk factor for neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. To

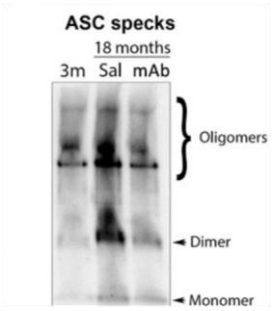
determine the potential of IC 100 to modulate age-related inflammation in the brain, male mice, aged 3 and 18 months old, were treated with IC 100 or saline intraperitoneally at 10 mg/kg and sacrificed 3 days later for analysis of their brain cortex.

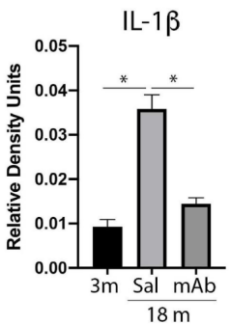
IC 100 significantly reduced cortical inflammasome proteins (NLRP1, ASC, Caspase-1), ASC Specks, and IL-1β ($p<0.05$).











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

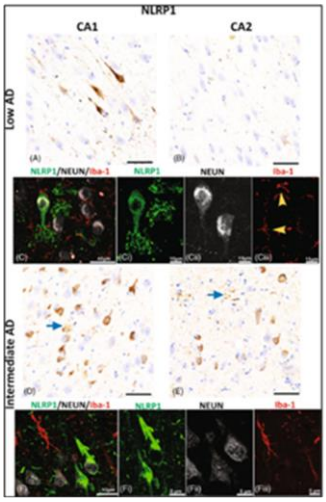
Alzheimer's Disease (AD)[7]

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

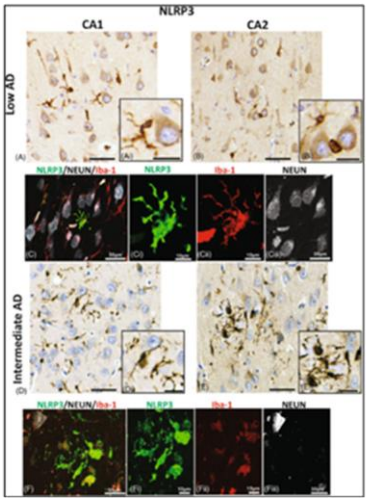
progression, a panel of commercially available antibodies were used to identify Aβ and pTau, and the inflammasome proteins, NLRP1, NLRP3, and caspase-1 in postmortem human brains with and without intermediate AD neuropathological changes. Additionally, IC 100, which targets ASC^{PYD} and a commercially 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 that multiple inflammasome pathways are associated with AD development. NLRP1 was expressed primarily in neurons and NLRP3 was expressed primarily in microglia.

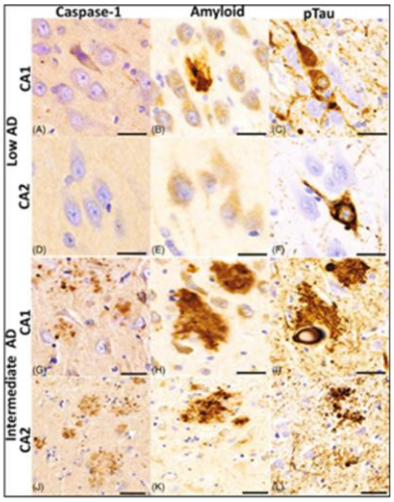
NLRP1 in Hippocampal Neurons



NLRP3 in Microglia

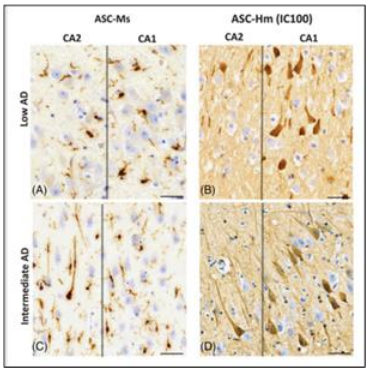


Caspase-1 in Hippocampal Regions



Labeled IC 100, targeting ASC^{PYD} demonstrated binding to ASC in neurons, whereas labeled antibody targeting ASC^{card} demonstrated binding to ASC in microglia. Data suggest that IC 100 can target neurons in the early stages of AD, and potentially reduce inflammasome activation.

ASC in Neurons & Microglia



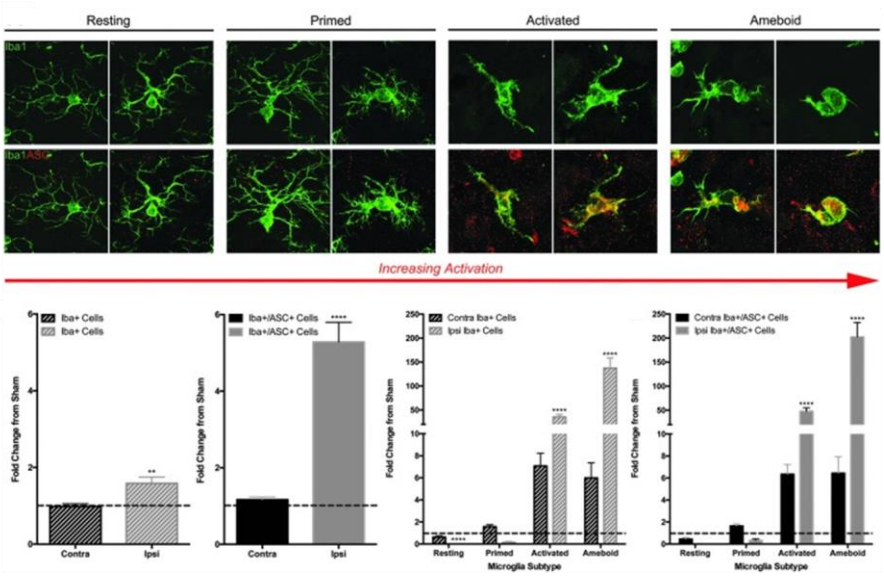
This study demonstrates that increased expression of multiple types of inflammasomes (NLRP1 and NLRP3) occurs early in AD, and that IC 100 binds to neuronal ASC. This supports the potential of Inflammasome ASC Inhibitor IC 100 as a therapeutic option for early intervention to potentially delay AD progression.

Penetrating Traumatic Brain Injury (PTBI)[8,9]

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 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.

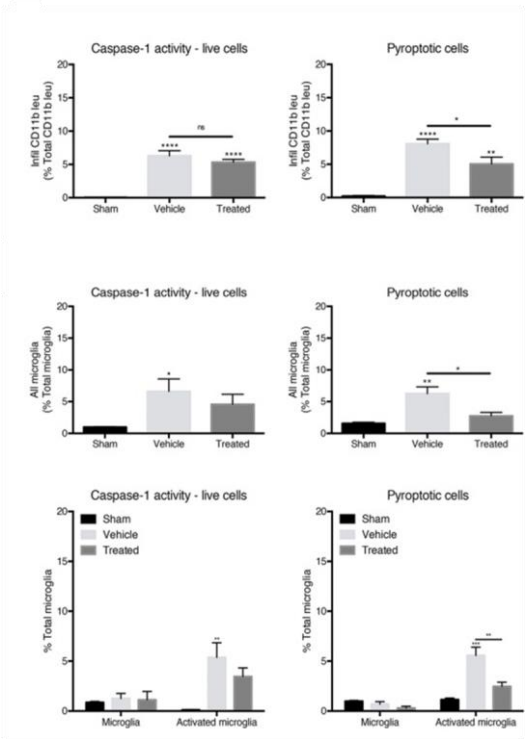
Inflammasomes were activated in microglia 48 hours following PBBI, as evidenced by the significant increase in number of activated and ameboid type microglia expressing ASC in the injured cortex. Inflammasome activation persisted for 12 weeks following injury.

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 Inflammasome Inhibitor IC 100.

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



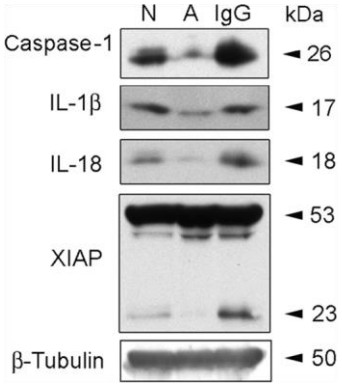
Spinal Cord Injury (SCI)[10]

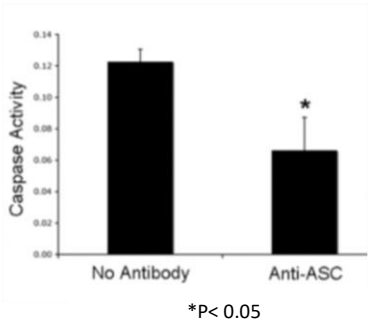
In the United States, there are approximately 17,000 new cases of SCI each year, and roughly 282,000 persons are estimated to be living with SCI. SCI is an insult to the spinal cord resulting in a change in the cord's normal motor, sensory, or autonomic function. A vigorous immune response mediated by 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.

Inflammasome Proteins

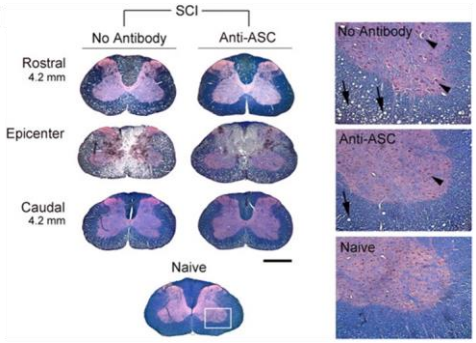


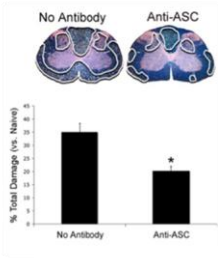


*P< 0.05

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

Histopathology

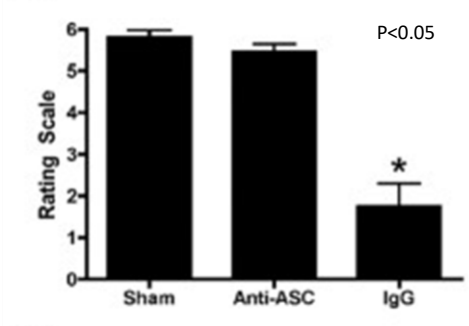




*P<0.05

Inflammasome ASC inhibition improved motor skills based on the sticker removal rating scale.

Sticker Removal Rating



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.

Summary and Conclusions

- Neurological diseases/disorders are the leading cause of physical and cognitive disability globally.
- Data suggest that activation of more than one type of inflammasome contributes to the damaging inflammation that is pathogenic in certain neurological diseases.
- A growing body of evidence supports the role of ASC and extracellular ASC specks in the progression of neurological diseases.
- ZyVersa's Inflammasome ASC Inhibitor IC 100 is in development to ameliorate heightened and prolonged inflammasome activation that perpetuates damaging inflammation underlying numerous CNS and peripheral inflammatory conditions. Because it inhibits multiple types of inflammasomes and extracellular ASC specks, it is expected to better control inflammation than other approaches.
- Data demonstrate that IC 100 crosses the blood brain barrier, and that it displays strong pharmacological signals in a variety of neurological conditions: multiple sclerosis, age-related inflammation, Alzheimer's disease, traumatic brain injury, and spinal cord injury.

Based on the data highlighted in this paper, it can be concluded that ZyVersa's Inflammasome ASC inhibitor IC 100 has potential to become a promising therapeutic option for numerous neurological diseases and disorders.

References

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