SAJE Pharma, LLC

GSNOR Inhibition: A Unique Platform for Neurodegenerative Diseases

Matt Bradley, Ph.D.
mbradley@sajepharma.com
301-332-0942

Company Objective

To be the leading company in regulating protein nitrosylation for the development of treatments for neurodegenerative diseases with multiple pathophysiological drivers

SAJE's Technology Platform Uses GSNOR Inhibitors to Regulate Protein Nitrosylation

- Protein nitrosylation, like phosphorylation, is a major regulator of cellular signal transduction pathways, that, when dysregulated, cause disease.
- GSNOR is the single enzyme that regulates over 90% of nitrosylation.
- Over-expression of GSNOR is seen in many inflammatory, oxidant-based, and fibrotic diseases in many categories including neurodegenerative.
- Inhibiting GSNOR leads to > 50 therapeutic benefits and activity in > 20 disease models.

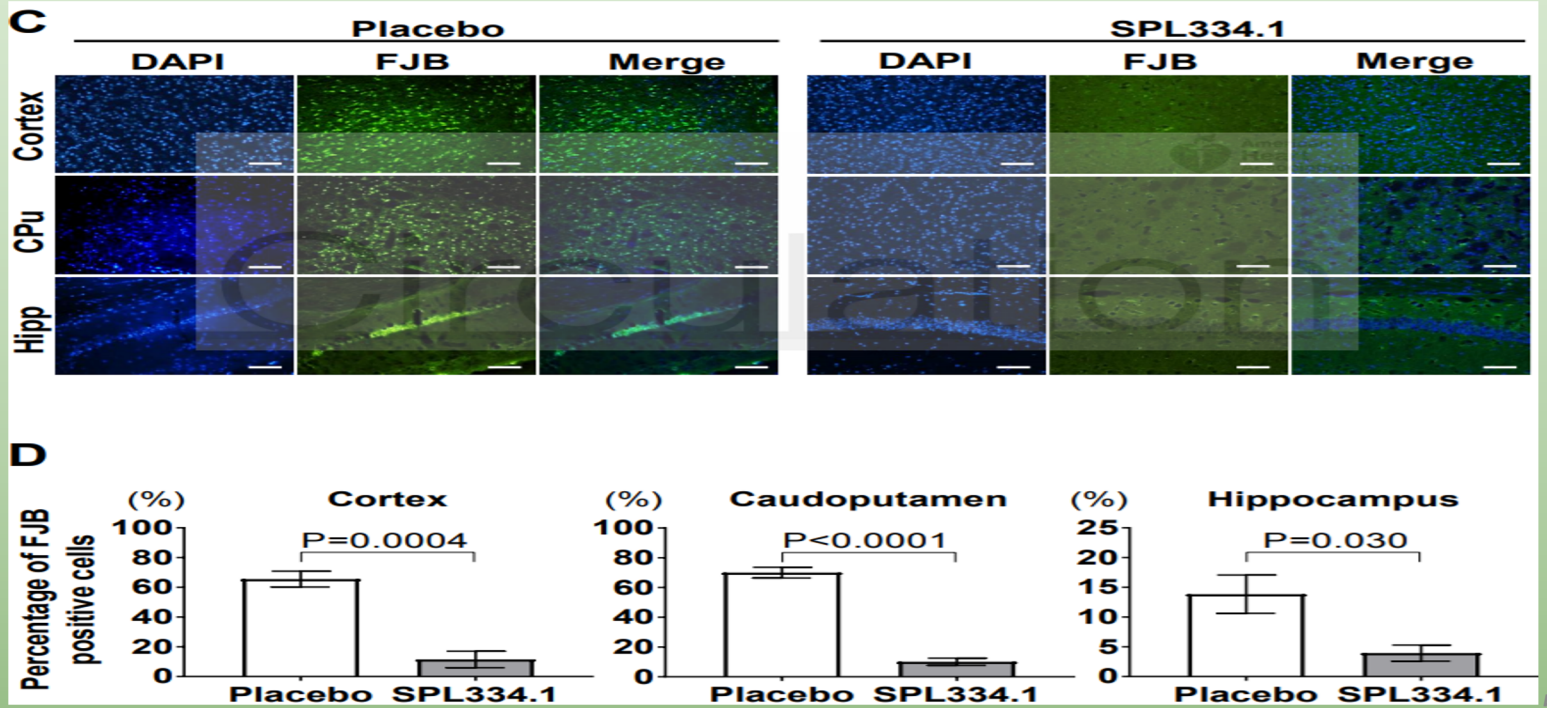
SAJE GSNORis Prevent Neurodegeneration After Ischemia & Ocular Pressure Overload

1. In a Cardiac Arrest/Ischemia Reperfusion model: Hayashida, et. al., Circulation. 2019;139:815–827 (see details below).

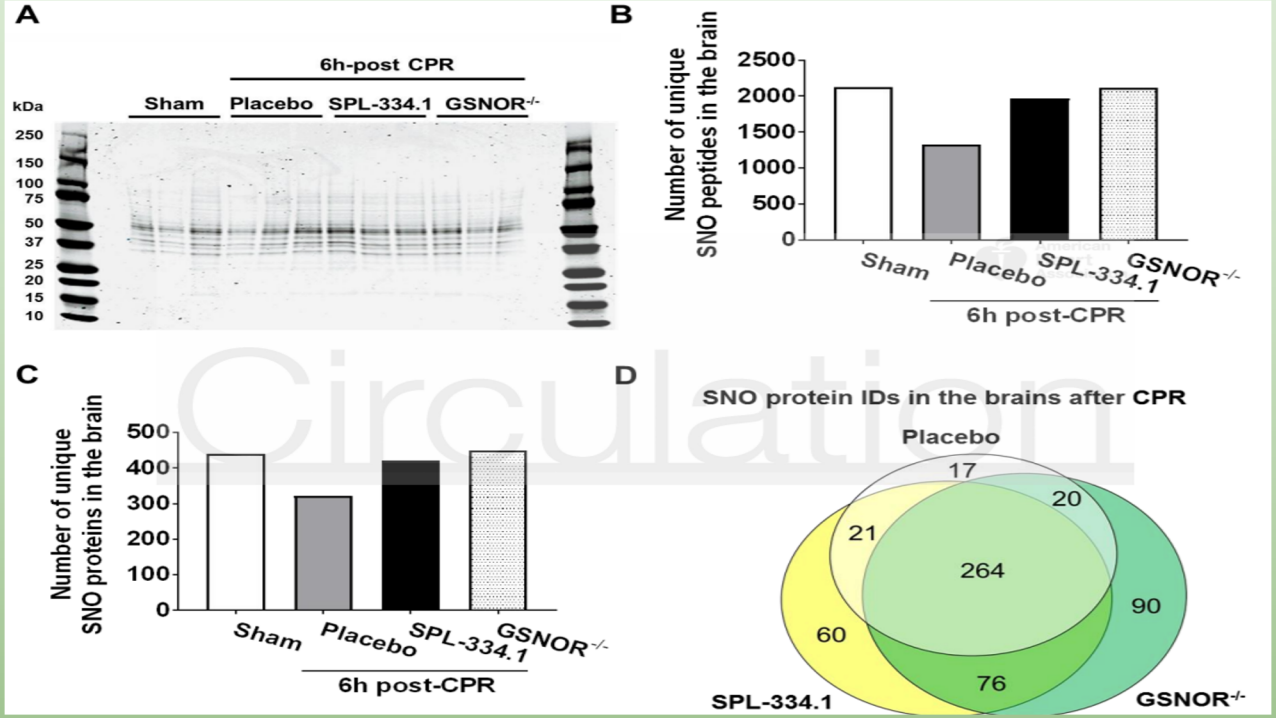
2. In a therapeutic retinal pressure overload model: GSNORi started 2 weeks after beginning microbead induced 24% pressure overload.

3. Results showed that SAJE's GSNORis prevented microglial activation, loss of anterograde axon transport, and retinal neuron degeneration in brain.

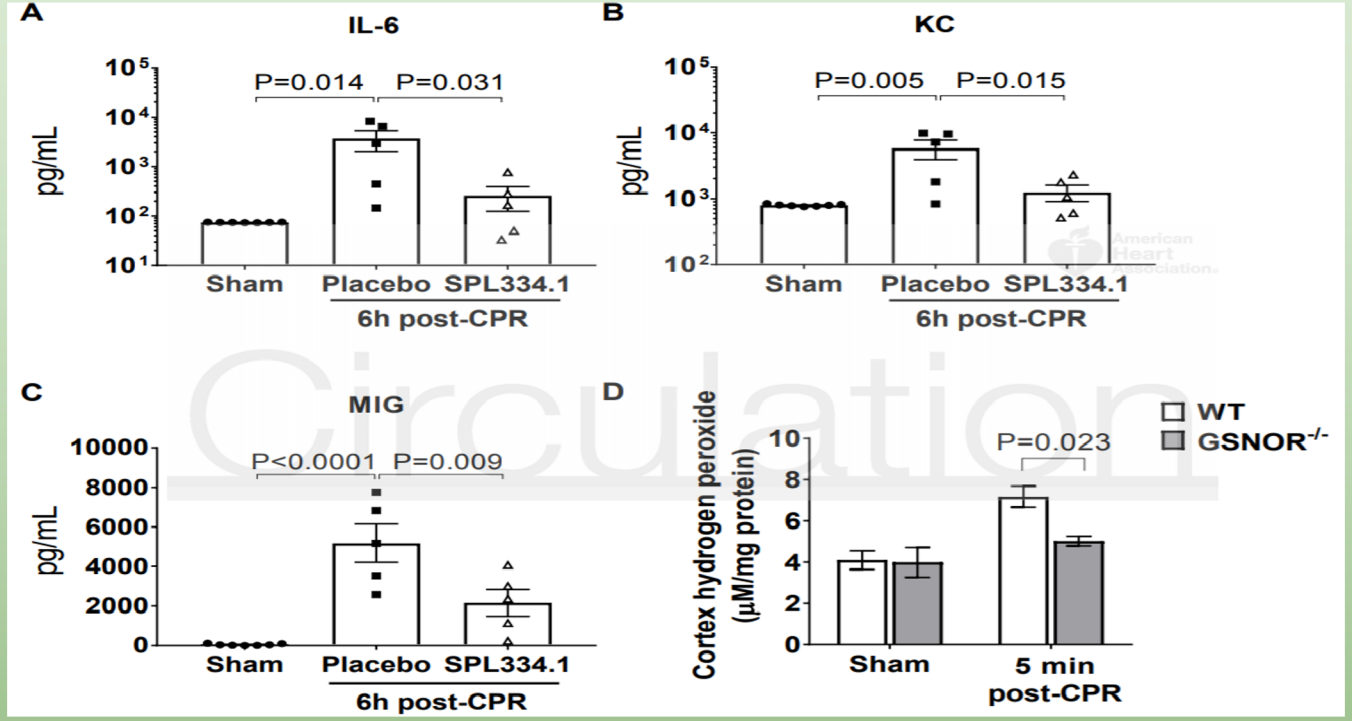
Hayashida, et. al., Circulation. 2019;139:815–827.
GSNORi improves brain cell survival



Effects of GSNORi on Brain Protein NItrosylation

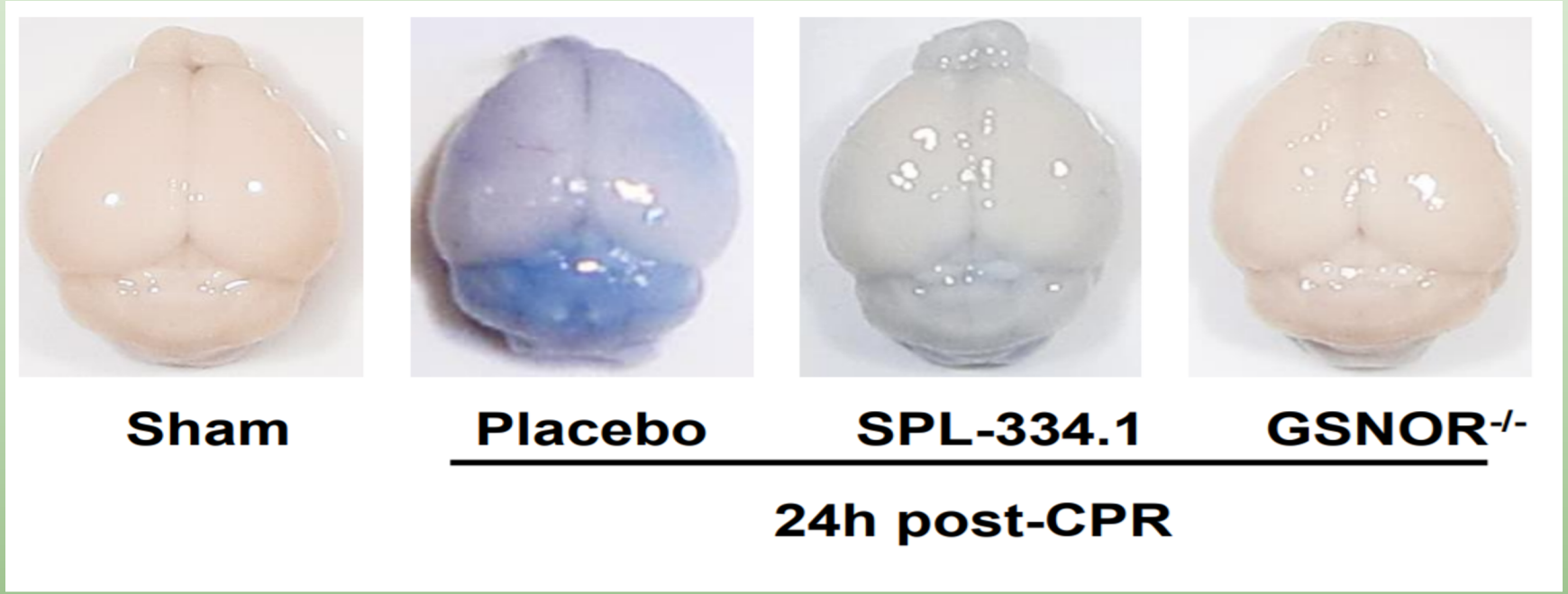


Effects of GSNORi on Inflammatory Mediators after CA/CPR

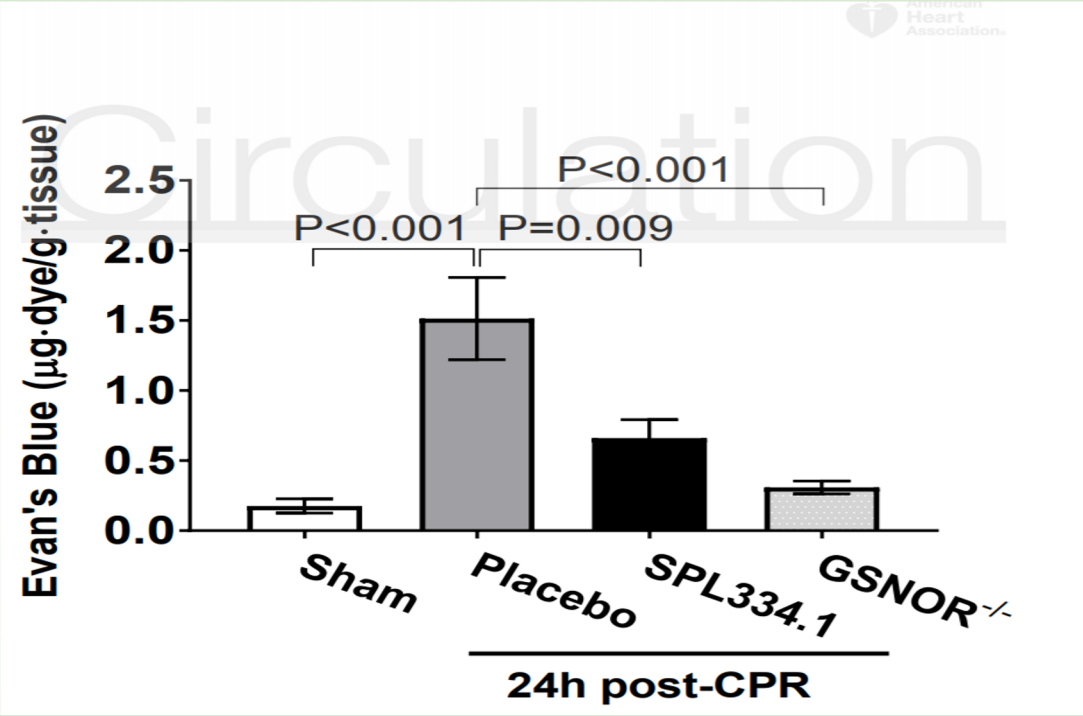


Neuronal Survival after CA/CPR

Evans Blue brain staining



Evans Blue Staining after CA/Ischemia



GSNORi Nitrosylates 10 Brain Mitochondrial Pathways

GSNO significantly increased the expression of:

- BDNF—brain-derived neurotrophic factor important in neuroregeneration
- TrkB—tropomyosin receptor kinase B, a receptor for BDNF, important for synaptic plasticity underlying hippocampal-dependent learning & memory
- pTrkB—phosphorylated TrkB
- PECAM-1—platelet endothelial cell adhesion molecule, removes aged neutrophils
- Myelin

GSNO significantly decreased:

- GFAP expression, glial fibrillary acidic protein
- Inflammatory cell infiltration into the brain

Literature Studies Supporting Roles of GSNO/GSNORi in Ameliorating Neurodegeneration: *TBI, EAE/MS, Vascular Dysfunction, Platelet Aggregation, Cerebral Hypoperfusion, pBCCAO, & Increasing Neurotrophic Factors*

(See SAJE White Paper for references)

GSNORi or GSNO in Multiple Sclerosis (MS)

Myelin Oligodendrocyte Glycoprotein (MOG 35-55) Model

(Multiple Publications)

- Both GSNO & GSNOR inhibitor, N6022, were tested in the experimental autoimmune encephalomyelitis (EAE) model of MS
- The results showed profound normalization of MS pathophysiology:

1. GSNO inhibited IL-6-induced STAT3 activation (Tyr705 phosphorylation) by S-nitrosylation of the STAT3 protein on Cys259
2. GSNO downregulated the IL-6 & TGF-β induced expression of RORγt, a TH17 specific transcription factor
3. GSNO inhibited the IL-6/TGF-β & IL-23 induced TH17 cell polarization and their effector functions
4. GSNO reduced proinflammatory transcription factors: NF-κB, AP-1, & STAT3 and thus modulates gene expression for various proinflammatory effectors, such as ICAM-1, and VCAM-1
5. GSNO inhibited endothelial recruitment of peripheral immune cells into the CNS
6. N6022 inhibited TH17 and expression of IL-10 in spinal cord derived T cells, one of the potential mechanism for immunomodulation and CNS protection

GSNO (and therefore GSNORi) in a Model of Traumatic Brain Injury

(Multiple Publications)

- GSNO reduced BBB leakage and improved neurobehavioral functions
- GSNO blocked infiltration of macrophages and reduced the expression of ICAM-1, MMP-9, and iNOS
- GSNO inhibited the TBI-mediated decrease in the expression of ZO-1 and occludin.
- GSNO improved tissue histology and decreased loss of both myelin and neurons in the brain
- GSNO reduced the oxidative injury in the neurovascular unit caused by peroxynitrite and its metabolites.
- A 2- week treatment with GSNO not only inhibited the loss of myelin and axons, but also enhanced the expression of neurotrophic factors (CNTF & BDNF). These findings indicate that GSNO not only down regulates TBI-induced neurovascular exacerbations but also stimulates the mechanisms of neurorepair.

GSNORi Prevents: Vascular Dysfunction, Platelet Aggregation, & Cerebral Hypoperfusion

(Multiple Publications)

- BP: GSNORi decreases blood pressure in hypertensive but not normotensive rats in a high salt model
- Platelet aggregation: GSNO decreases leukocyte adhesion to endothelium and platelet aggregation (used to coat cardiac stents)
- Vascular dysfunction: GSNORi increases vascular function in a diabetic mouse model
- Cerebral Hypoperfusion: GSNORi increases perfusion in models of heart failure, stroke, & hypertension

GSNO (and thus GSNORi) Effects in Rat Permanent Bilateral Common Carotid Artery Occlusion (pBCCAO) Model

GSNO significantly increased:

- Memory and learning

GSNO significantly decreased in brain:

- Amyloid β accumulation
- Tau hyperphosphorylation
- Neurodegeneration
- ICAM1
- VCAM1
- NfKB
- GSK3 β & Cdk5 pathways
- Calpain/p25/Cdk5 pathways
- STAT3
- iNOS & nitrotyrosine expression

GSNORi Increases Neurotrophic Factors after Middle Cerebral Artery Occlusion Stroke Model

GSNO significantly increased the expression of:

- BDNF—brain-derived neurotrophic factor important in neuroregeneration
- TrkB—tropomyosin receptor kinase B, a receptor for BDNF, important for synaptic plasticity underlying hippocampal-dependent learning & memory
- pTrkB—phosphorylated TrkB
- PECAM-1—platelet endothelial cell adhesion molecule, removes aged neutrophils
- Myelin

GSNO significantly decreased:

- GFAP expression, glial fibrillary acidic protein
- Inflammatory cell infiltration into the brain

Safety of GSNOR Inhibitors

No mechanism based toxicity in preclinical safety or 400 pt Phase II clinical trials

SAJE's SPL-850 and 891 show no toxicity in efficacy and safety studies